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FOURTH ANNUAL REPORT ON CACAO RESEARCH, 1934.

A.—BOTANICAL SECTION.

THE BOTANICAL PROGRAMME OF 1934.

By E. E. Cheesman.

Propagation.

The stage is now rapidly being reached at which our selected cacao trees and the various methods of propagating them must alike be tested by a field experiment on a large scale. Such an experiment will demand large quantities of planting material, and the preparation of that material has been in consequence a main pre-occupation of the year's work. In order to ensure an ample and regular supply of suitable cuttings and good budwood from selected stock and scion types, nurseries have been laid out. The nurseries will accommodate 88 chupon stools of each of our nine standard stocks, and 22 stools (11 chupon and 11 fan) of each of the 100 selected scions, and are expected to provide all that is necessary for the subsequent propagation of about 2,000 trees a year. The immediate task is thus to fill these plots.

Unfortunately the weather could scarcely have been less favourable, and it has to be admitted that the routine propagation has dropped rather badly behind the provisional schedule. Following on the excessively wet weather of 1933, 1934 in Trinidad was abnormally dry, and the rains which usually come in May were delayed until September. The drought delayed the planting out of rooted cuttings, and the growth of such as were out beforehand, but its most serious effect was on the quality of cuttings available, so that excessive leaf-fall took place in the propagators and rooting percentages were low.

Mr. G. E. L. Spencer of the Botanical Department of the College took over the routine propagation when Mr. Pyke went on leave, and thus bore the brunt of the worst months, and congratulation and thanks are due to him for contriving in spite of the difficulties to produce several hundred plants before the end of the year. It is expected with some confidence that once the nurseries are fully established the main difficulties of bulk propagation will be over. We should then be able, by control of shade and watering, to produce just the right kind of growth for successful rooting, but so long as we are dependent on the original trees for supplies, progress must depend very largely on weather conditions.

Arising partly from the scarcity of good cutting material, considerable attention was devoted to budding. Budwood was brought in from a number of the I.C.S. trees and successfully worked on to seedling stocks, and the budlings will help on the programme this year. At the same time, budding was practised in connection with the testing of I.C.S. and other trees for resistance to Witches' Broom disease. The interest of some practical planters in this method of propagation has led Mr. Pound to put together notes on his experiences of the technique, which appear in this Report.

The absolute necessity of increasing productiveness to counter falling prices is becoming generally recognised, whilst our selection programme has re-emphasised the low efficiency of the average seedling tree. Replacement of the poorest of the existing trees is therefore to the fore, and there are some prepared to go further than the use of selected seedlings if a practicable method of vegetative propagation is available. The use of cuttings must of necessity remain in the experimental stage for some time longer, but budding on seedling stocks is an established method, relatively simple and relatively cheap, and probably intermediate between seedling and cutting propagation in its advantages. This is not the place to elaborate the *pros* and *cons* of budding as an estate method, but at the least it merits fuller trial on the plantation scale than it has yet received.

Selection.

Mr. Pound presents descriptions of a further 18 trees added to the I.C.S. list, and expects to complete the selection programme during the current crop. It will be noted from his remarks that, in spite of an adverse season, the 1933-34 crop from most of the trees described last year came well up to expectation or surpassed it, and none failed badly. Some others which were held back for further observation had to be discarded on actual yield, though the evidence indicates that the fault was in conditions rather than in the tree. These facts show that the standard adopted is sufficiently rigorous, and must be regarded as highly satisfactory.

Studies of Fruitfulness.

Preoccupation with selection and propagation has not precluded advances along other lines. Our knowledge of fruit-setting has been carried a step further by Mr. Pound's discovery of what he calls "conditional self-compatibility" in cacao trees. This means that although, as previously reported, many trees set poor crops on account of self-incompatibility, it is possible in some cases at least for the self-incompatibility to break down under changed external conditions. The implication is that we may be able to learn how to break it down by horticultural methods, and thus to extend the setting period with advantage to the crop. For the moment this is a complication, rather than a simplification, of our views on setting, but it opens up new potentialities of increasing yield on established plantations and represents a real advance in fundamental knowledge.

The study of ovule loss at fertilization, a very painstaking piece of work, has shown that the discrepancies between ovules per ovary and beans per pod spring from two causes. There is a certain loss fairly constant for any particular combination of parents, which seems to be due to genetic factors and reflects again on the complex ancestry of cacao trees. There is also a heavier but irregular loss occurring only in occasional pods, and probably traceable to pollination factors. There appear no immediate economic applications of this information, but it adds another detail to our picture of the building up of yield in cacao, and thus has intrinsic value.

The mineral nutrient experiment at River Estate, described last year, has enabled preliminary analyses of the effects of manures on such characters as bean weight and bean number, used in selection, and on setting, wilting and disease incidence important in studies of fruitfulness. The results on one year's figures are only tentative, but interesting. This experiment has also provided a check on previous conclusions with reference to lay-out and calculation of precision of results. It has further furnished material for leaf analyses by the Chemical Section.

Other Work.

Relieved of routine propagation, Mr. Pyke on his return from leave was able to turn his attention to other physiological problems. His investigation of viability of cacao beans after storage was carried out in collaboration with the officers of the College Low Temperature Research Station and at the suggestion of the Kew authorities. The object is to facilitate the carriage of pods over long distances, as will be necessary when it is desired to introduce disease-resistant or superior types from one country to another. It is essential if the full potentialities of botanical research are to be realised that there shall be interchange of types, subject of course to strict quarantine precautions, between one country and another, and methods of ensuring viability over the time taken in transit must therefore play an important part in general progress.

Mr. Pyke's other investigation needs perhaps rather more explanation. It is well known to botanists that the roots of many forest trees have associated fungi within or upon them which modify their feeding process. In the early stages the fungus is apparently parasitic on the root, but later on it dies and is digested by the root cells. Nitrogenous and other substances contained in the fungus thus seemingly become available to the tree by a process essentially different from the normal uptake through the root hairs, and in studying the nutrition of the tree it is important that the difference should be recognized. Roots associated with fungi in this manner are technically known as *mycorrhizas*. Cacao being ecologically a forest species, we have long realised the possibility that careful examination might disclose mycorrhiza in its roots, and at the first opportunity a preliminary search has been made. Mr. Pyke has found that there is quite commonly in cacao roots a fungus which in appearance and distribution closely resembles mycorrhiza fungi described by other observers in other plants. Nothing further can be said at present; it remains to be proved that this fungus is of any benefit to the roots which it inhabits, and further investigation is likely to prove intricate. The borderline between symbiosis and parasitism is in any case an ill-defined one, and the technical difficulties to be overcome before any physiological rôle can be attributed to suspected mycorrhiza are formidable. Nevertheless the subject is of profound importance and will need to be very carefully followed up.

Erratum.

In the Third Annual Report on Cacao Research, 1933, p. 16, Col. 1, line 34, for "F. Budded by method B. No shade" please read "F. Seedlings. No shade". We regret this slip, and hope that it will have been clear to most readers from the context that F is a seedling block.

NOTES ON THE BUDDING OF CACAO.

By F. J. Pound.

Introduction.

The first successful cacao buddings seem to have been made by Harris in Jamaica in 1903, and by Heyl in Java at about the same time. Subsequently success was reported in Haiti (1906) the Philippines (1911) Trinidad (1913) Dominica (1915) and Surinam. Further historical details and literature references may be found in a review by Cheesman (1). The operation is therefore by no means new, yet from numerous enquiries received from interested growers it appears that a few notes on it may be welcomed in this Report. It happens also that during 1934 a good deal of attention has been devoted to budding as an adjunct to other investigations. In the collection of nursery material from selected trees, for example, it often happens that the only shoots available are unsuitable for direct propagation as cuttings. The most economical course is then to collect budwood, transfer the buds to seedling stocks in our nurseries, and take cuttings later from the budlings instead of returning to the original tree. Furthermore, budding has been employed in connection with an experiment designed to test the resistance of I.C.S. and other trees to Witches' Broom disease (*Marasmius perniciosus* Stahel) and for one reason or the other some thousands of buddings have been made.

The opportunity was taken to plan these routine buddings in such a way as to extract information from them on points of technique, and several observations were made that seem hitherto to be unrecorded. These have been incorporated into the detailed account of procedure and to some extent justify its inclusion in a research report, though for the basic method no originality is claimed.

Differences in Method.

Methods used by previous workers fall for practical purposes into two classes, viz.: those in which fan buds are used on small stocks and those in which chupon buds are used on large stocks. Most of our own work, like most of the previous Trinidad work, has been done with fan buds on small stocks, and this method, which is also the simplest, will be described in detail. The use of chupon buds will be discussed afterwards.

Using small stocks (which means seedlings about one year old or less) further variation in procedure is possible according to whether they are budded (*a*) in pots, (*b*) in a plot nursery for subsequent transplantation, (*c*) at stake. The third method, however, scarcely merits mention under this heading, because if budding is to be done at stake there are advantages to be gained from letting the stock grow large and using a chupon bud. Between the pot and the ground nursery, choice must be made on a balance of advantages. We have used both, and there can be little doubt that it is easier to grow vigorously healthy stocks in the ground than in pots, especially the bamboo joints most likely to be employed, and hence easier to obtain a high proportion of successes. On the other hand, the potted plant is more convenient to handle, both in the actual budding and in the subsequent planting out. If bamboo pots are chosen, they must be large joints affording good root-room.

In application of the bud we have used exclusively the rectangular patch method worked out by the Trinidad Department of Agriculture and also mainly used elsewhere. Stahel (2) uses an oval patch on larger stocks, and T-buddings have also been made with success by some workers.

Technique with Fan Buds.

A suitable bud is selected and removed together with the bark surrounding it as a rectangular patch about $\frac{1}{4}$-inch wide by about $\frac{3}{4}$-inch long. To accomplish this, two vertical cuts are first made on either side of the bud, longer than the patch is to be. The two horizontal cuts are then made deep, equidistant from the bud above and below it, intersecting the vertical cuts and going well beyond them. The patch can then be levered out quite easily without splitting. It is at once applied to a suitable position on the stock and held there as a pattern whilst two horizontal cuts, just down to the wood, are quickly made in the bark of the stock immediately above and below it. As far as our experience goes, it makes no difference whether the position selected is already the site of a bud or not. Two vertical cuts follow and the patch of bark from the stock is then levered out and the bud patch inserted in its place. The vertical fit of the bud patch into the "window" made on the stock must be close, hence the use of the patch itself as a pattern. More laxity is allowable in lateral fit, and for speed of working the vertical cuts in the stock may therefore be made slightly wider apart than the patch requires. Any gap that may be left at the sides of the inserted bud-patch will rapidly fill with callus tissue.

The next operation is to bind the bud into place with waxed tape $\frac{1}{4}$-inch wide (Plate 1, Fig. 1). The actual method of tying is best worked out by experience by each individual operator, the important points being that the "eye" is left uncovered, and that the rest of the patch is completely covered, securely enough to prevent entry of water. The easiest method is probably to start with the centre of the tape over the upper horizontal cut, and to work in a criss-cross manner around and down the patch. Above the eye the tape is pulled tight, but this is not necessary below.

(1) Cheesman, E. E.—"The Vegetative Propagation of Cacao." *Empire J. Exp. Agr.* 2 (1934) 40-50.

(2) As described by van Hall, "Cacao" (1932).

The tape is waxed before use in order to make it both water-tight and slightly adhesive. It is cheaply prepared by dipping plain cotton tape into hot beeswax to which a little antiseptic such as creosote has been added. More elaborate and expensive mixtures have been used with no better success. The tape is cut into 10-inch lengths and stored until needed. Just before application each piece is drawn rapidly once or twice between the fingers to make it adhesive. Tests by deliberate watering over the bud patch show that if properly applied this tape effectively excludes water from the part covered, and this is important, for once water reaches the cambial tissues from outside, union of bud and stock is prevented.

Quite often the "eye" sprouts within a few days of insertion, but in some cases sprouting may be long delayed. The tape can possibly be removed at seven to nine days, but as a rule it is left for 14 days for safety. Tape can be used a second time.

Subsequent treatment of the budded stock depends on the period of dormancy of the bud and on the weather. Generally, as soon as the bud shows signs of sprouting the stock can be topped back, leaving only a few leaves (Plate I, Fig. 2). Stocks budded in bamboo pots should not however be cut back to the final stage shown in Plate II, Fig. 3, until the budling shoot is well grown. In the plot nursery, cutting back can be done much more rapidly, except in the driest weather, when the budling is in danger of drying out. Dormant bud patches often fail to sprout at all until the stock is cut back severely, and this can usually be done without risk if the patch is well healed on to the stock.

This is the whole of the basic technique, and it will be seen that budding is a very simple operation. There are, however, a number of points to be watched if a good percentage of successes is to be consistently obtained. They concern chiefly the choice of suitable stocks and suitable buds, and will be discussed under those headings.

Choice of Stocks.

Stocks extensively used last year were seedlings about 12 inches high, with a diameter at the base equal to that of an ordinary lead pencil (about $\frac{1}{3}$-inch). A much smaller diameter would be inconvenient, but when stocks are budded in pots it is well to carry out the operation as soon as possible so that they shall not become unduly potbound. In the ground they may be left until larger, but must not become too large for convenient transplantation. As to age, presumably the sooner they attain the right size the better, since rapid growth connotes health and vigour; at all events we have found no evidence of any direct age effect within reasonable limits. There is little knowledge to guide selection of parents for stocks, and in practice any good, hardy, heavy-bearing tree is generally taken.

General health is very important. A leaf-edge browning is common at times among cacao seedlings in bamboo pots, and is probably due in the main to irregularities in water supply, as it is especially noticeable at times of atmospheric aridity. During the dry summer of 1934 this gave considerable trouble and was associated with attacks of several weakly parasitic fungi. Until it was overcome, in some cases by transplanting into better soils and in others by addition of potash and lime to the pots, successful buddings dropped to a very low figure. Light too appears to have some influence on the degree of success obtained, probably through its reaction on the general health of the stocks. Of two sets of stocks kept in a concrete room with lateral light only and a still atmosphere, the first set of 100 buddings gave 49 successes, and the second of 410 gave 215 successes (52 per cent.). Of 100 buddings performed at the same time on stocks kept in a well lighted greenhouse 65 were successful, and from the controls out of doors, lightly shaded, there were 66 successes. The inference is that best results are not obtained from stocks growing under conditions of too heavy shade necessary to keep humidity high during the dry season in Trinidad.

With these facts in mind, tests were made of pretreatments of stocks in pots with inorganic manures. Earlier experiments on growth rates of manured seedlings had demonstrated that cacao plants in bamboo pots can tolerate only very small quantities of inorganic salts at a time, and the amount applied to each pot at each dressing in the present experiments was as much as would cover a threepenny piece. Three applications were made, at weekly intervals, starting one month before budding. Treatments were arranged as a complete N, P, K trial, with 20 stocks to each of eight treatments. Budwood was used from four different trees, and practically the whole of the variability of the results was due to the variability in this budwood.

Analysis of Variance.

	D.F.	Sum of Squares.	Variance.
Between Scions	3	88.5	29.5
Between Treatments	7	6.0	0.86
Error	21	20.5	0.98
Total	31		

Thus the addition of inorganic salts to stocks already growing in fairly rich compost does not appear to give any advantage, at least when carried out only one month before budding.

It has been the custom not to discard stocks on which buds failed to take, but to rebud them when of suitable size by inserting a fresh bud higher up. Poor results were usually obtained

PLATE I.

FIG. 1.—*Showing bud bound into position with waxed tape.*

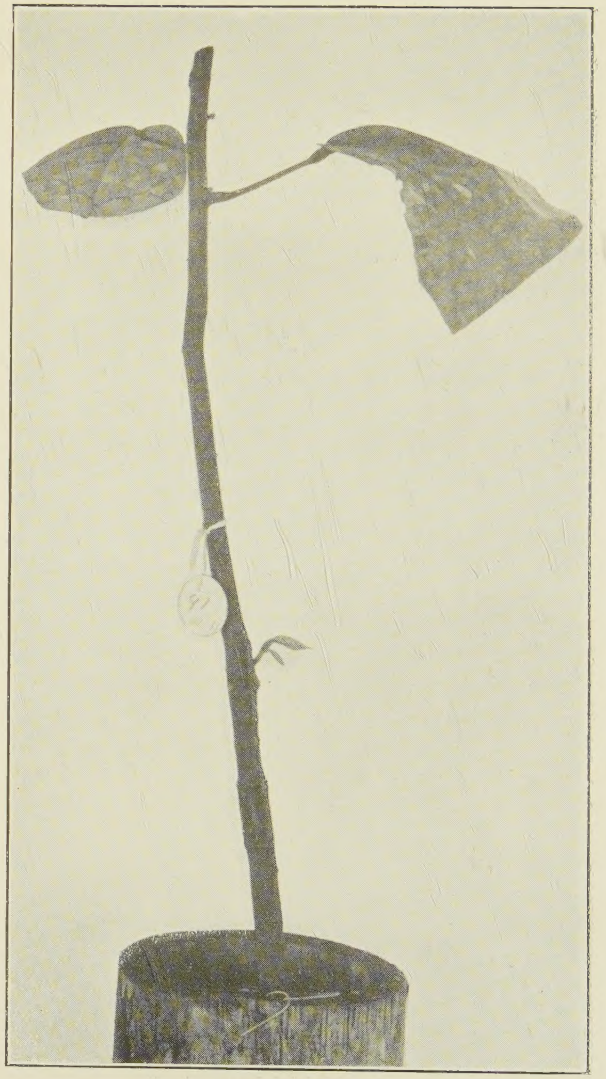

FIG. 2.—*Bud sprouted; stock topped.*

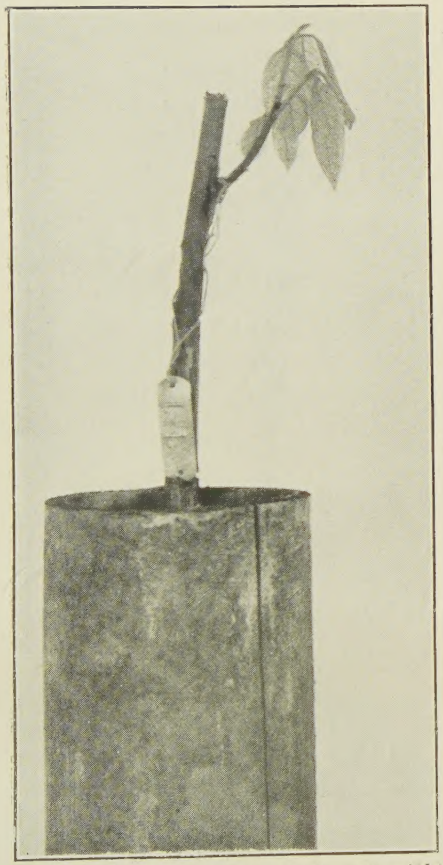

FIG. 3.—*Later stage; stock cut right back.*

FIG. 4.—*Apparatus for control of pollination in cacao.*

from such second buddings, although in individual cases they might be very good. The following details of comparable sets of buddings illustrate the effect :—

Date.	No. buds applied.	Nature of Stock.	Per cent. Take.
14.8.34	60	fresh	60
17.8.34	135	fresh	50
21.8.34	130	once budded	15.4
22.8.34	100	once budded	6
23.8.34	10	twice budded	0
24.8.34	52	fresh	86.5
28.8.34	150	fresh	66.7

There is evidence to be given later of stock-scion incompatibility in certain cases, and it may be that those stocks that failed to take a bud on two or three occasions were genetically unsuited to unite with the buds applied. On the other hand the failures may have been due on both or all occasions to the physiological condition of the stocks. One set of once-used stocks, after a comparatively long "rest" gave 200 successes from 400 specially chosen buds that were applied, whence it appears that if failures are to be rebudded it may be advisable to leave them for some considerable time before making the second attempt.

Choice of Budwood.

The budwood used must be of approximately the same diameter as the stock on which it is to be placed. Since the earliest attempts at budding cacao it has been recognised that the bud appears to be in the best condition for use as soon as the subtending leaf falls. The bud-patch can then usually be removed easily without fear of splitting, particularly if there is a flush of growth commencing at the end of the twig. The number of suitable buds, even on mature trees, is often very few, and attempts were made to find means of using buds before the fall of the subtending leaf. Poor results were obtained, even though the leaf petiole was severed close to the bud and the wound treated with an antiseptic. Probably these buds are too immature for success. It was discovered, however, that they may often be matured artificially if the leaf is removed by a cut through the middle of the petiole. After a week or ten days the stub abscisses, and the buds are then in a particularly good condition. Most of our best results have been obtained from budwood prepared in this manner, and it is a simple operation for budwood nurseries when large numbers of buds are required from one scion type.

Older budwood can be used with success in some cases. Its chief drawback is that the primary bud has usually been lost, and unless a secondary bud has been formed a long period may elapse before sprouting takes place, even though the union with the stock may be good. Another feature detracting from the value of old budwood is the frequent presence on the outer layers of bark of weakly parasitic fungi. These do no visible harm until the twig is wounded, but may grow rapidly in the patch after the budding operation. During 1934, when the abnormally dry conditions led to low vitality in trees in certain fields, fungal breakdown of the bud-patch tissues under the tape was common even with younger budwood, and in this case treatment with formalin was found beneficial. The percentages of successful buddings after immersion of the budwood in various strengths of formalin for varying periods were as follows :—

Time of Immersion.	Per cent. Formalin.			
	0	½	1	2
1 min.	12.5	40	40	40
3 min.	17.5	75	73	52.5
5 min.	—	62.5	70	60

Thus surface sterilization by immersion in ½ to 1 per cent. formalin for three to five minutes may be very useful under certain conditions, though it is not suggested as a general routine measure with normal budwood.

It is often necessary to transport budwood from distant estates, and transport even between different countries may sometimes be desirable. Our experience has shown that considerable loss of viability occurs after 36 or 48 hours unless great care is taken to keep the twigs wrapped in damp cloth or otherwise protected. In transport over periods of a few hours it has been found satisfactory to collect the budwood in lengths of less than 12 inches and wrap immediately in cool tannia (*Xanthosoma*) or plantain leaves. In dry weather it is still better if the bundles so obtained can be immersed in water for a few minutes before the journey. More elaborate methods, such as wrapping in damp sterilized moss, gave no advantage if the buds were applied early on arrival at the nursery—in fact in tests, buds transported in this manner gave fewer successes. With all precautions there is some loss of viability if the buds are not applied early. From relatively distant estates sufficient budwood has often been collected on one visit to keep the budding staff employed for two days. That used on the first day has been taken at random as required from a humid chamber (propagator) and the remainder left there overnight. The following table shows results from the first day's buddings of seven consecutive sets, compared with those from buddings on the second day from the same material :

	Per cent. Successes.						
1st Day	37.3	25.0	40.8	18.0	60.0	50.0	53.3
2nd Day	24.0	16.1	25.0	8.8	29.0	21.0	38.9

Each figure is the mean result from ten buds of each of ten different trees, and between the sets of ten considerable variation occurred, but the general result shows clearly the deleterious effect of keeping budwood before use during the very dry weather of 1934.

As to the upper limit of duration of viability, it may be noted that a few buds have taken from very carefully selected and packed budwood after a week, but the problem of retaining viability over longer periods is sufficiently important to warrant further trials when sufficient clonal material is available.

Compatibility.

An outstanding feature of the routine buddings made during the year was the variability of the results. A large number of trees were used as scions, and as a rule ten buddings (sometimes 20) were made from each tree. As the results of each day's buddings were reviewed it was always found that the same operator, using comparable stocks, would have varying degrees of success with the different sets of budwood he had handled, ranging sometimes from no unions at all to the full possible ten. Part of this variability was traceable to the condition of the budwood, some trees giving plenty of buds regarded by the operator as ideal and others giving few. Part of it however remained even in cases where the budwood was to all appearances ideal, and from one tree as many as 30 apparently " ideal " buds have been applied without a single success, under conditions where at least 90 per cent. might be expected. Such results indicate some kind of incompatibility between stock and scion, which is no new phenomenon in horticulture. That " incompatibility " may be partly physiological is indicated by a general tendency of young trees to give better budwood than old, and 100 per cent. success has frequently been recorded with buds of young vigorously growing trees. That it may also be in part genetic is very probable, and further effects of it may be found even after union has taken place. Some patches with swollen buds fail to sprout at all, and others grow slowly, producing chlorotic leaves and a dwarfed habit, ultimately dying. From good, compatible buds, on the other hand, shoots four to six inches long have been obtained within six weeks of budding.

When clonal stocks are available in quantity this incompatibility will be further investigated, and it is likely that specific combinations will be found necessary for the best results.

Chupon Buds on Large Stocks.

The use by some workers of large stocks, one inch or so in diameter, originated in a desire to work over old trees by budding on vigorous chupons from their bases. The method has the advantage of utilising a well-established root system, and growth of inserted buds is extraordinarily rapid. Williams (3) reports rates of six inches a month, in trials of the system in Trinidad. In spite of this the results of the Trinidad experiments were unsatisfactory, on account of a tendency for the chupons themselves, at a later stage, to break away from the original parent trunk. This will be less liable to occur in the fortunate cases where the chupon arises at ground level and quickly forms its own roots, but the difficulty is to get shoots at the right level from every tree to be worked. Nevertheless the writer has seen this system applied with success on one estate where several hundreds of trees have been converted.

Since the curvature of the bud patch must fit that of the stock for good contact, budwood is required of the same diameter as the vigorous water-shoots used. Such budwood is most easily obtained by pruning the mother tree severely and causing it to produce vigorous new growth, among which will be similar chupons. The association of chupon buds with large stocks was therefore most probably accidental, in the first place, but their use profoundly affects the habit of the budded tree, which in this case has a true trunk and jorquette and closely resembles a seedling. The same effect can be obtained by growing large seedling stocks at stake and using similar buds on them instead of upon chupons. When this type of budding is done on a large scale, a necessity is the establishment of a special budwood nursery, in which budlings are kept stooled back to induce the formation of a regular supply of suitable chupons.

Chupon Buds on Small Stocks.

There is no reason to suspect differences in bearing abilities between fan budlings and chupon budlings, and the former can be trained to form a balanced tree, but it must be understood that if true trunks and jorquettes are desired chupon buds must be used. The resulting trees will be easier to train and will no doubt be preferred by some. It is therefore worthy of mention that the use of chupon buds does not necessarily involve the use of large stocks, and a technique is now being worked out to combine the convenience of pot or nursery budding of small stocks with the advantages of the chupon habit. The problem is to ensure a constant supply of " hard " chupons of small diameter, which are uncommon under field conditions. This can almost certainly be solved in a budwood nursery by keeping chupon stools of the required scion types hard pruned, the actual diameter of the budwood being regulated by the amount of pruning. The stools can be established in at least three ways. First, if the mother tree has suitable chupons these can be rooted as cuttings. Secondly, if only fan buds are available, fan budlings cut hard back at about a year old will usually give some chupons from the stub. The third possible method, which may be quicker than the second, was discovered only last year, when in order to hasten growth of fan buds a trial was made with ten stocks looped prior to budding

(3) Williams, R. O.—" The Budding and Grafting of Cacao." *Proc. Agr. Soc. Trinidad and Tobago,* 19 (1919) 294-301.

as described by van Hall (4) and fan buds were inserted at the apex of the loop, facing upwards. Not only did nine buds catch of which eight grew rapidly but five of the eight proved to be chupon shoots. The buds all came from one mature fan twig from which the leaves had abscissed naturally. The experiment was repeated with 47 prepared but rather advanced fan buds, 46 caught, about 40 grew rapidly and of these six had the orthotropic habit of chupons. Further information concerning the production in quantity of chupon budwood of small diameter will be forthcoming later from the experiments now in progress.

(4) Cacao, 1932.

Summary.

The established technique of budding cacao, using fan buds and small seedling stocks, is described in some detail for growers who may wish to give it a trial.

Details arising from experience of the method are incorporated, and in particular the characteristics of suitable stocks and budwood are described.

There is some evidence of stock-scion incompatibility, affecting results in some cases after all precautions had been taken to ensure success.

The use of chupon buds, which give a tree of seedling habit, is briefly discussed.

THE PROGRESS OF SELECTION, 1934.

By F. J. Pound.

In the Third Annual Report, short descriptions of a number of selected trees were given. It was pointed out in the preface that trees other than those described were on the selection list but owing to abnormally adverse weather conditions during 1933 many of them failed to hold the requisite number of pods and descriptions were withheld for the time. Abnormally heavy rain continued over the setting periods of the 1933-34 crop and caused very heavy losses of fruit in some cases in this year too. In the majority of instances the trees did eventually pull through. Those which failed a second time were removed from the list and either put back on the general observation list or discarded entirely. Whatever pods were produced did not depreciate in value. Beans in all cases were well up to standard. In a great many cases the actual loss was accidental, rarely due to a fault of the tree. Conditions for a short period would favour flowering and setting. As soon as cherelles reached a length of about one inch either a leaf change occurred if it did not rain, or the rains were too heavy. In either case the cherelles just wilted and died. The poor crop of many estates was due entirely to this chain of unfortunate circumstances.

Such facts coupled with recent discoveries in the field of pollen compatibility make it evident that the actual number of pods which a tree produces may be very little indication of its true bearing potentiality. Pod value appears to be a more certain characteristic.

For practical purposes trees which mature the greatest number of pods per annum alone have to be taken for our selection lists. This does not mean that a medium bearing tree which produces exceptionally fine beans may not in the near future be improved into a possible good bearer especially if for considerable periods during the year it is self-compatible.

The behaviour of some of the selected trees described in the last report is interesting. I.C.S. 1 was robbed very severely for cuttings and budwood yet in spite of this fact it produced 90 pods. I.C.S. 2 produced practically the same number of pods as I.C.S. 1 and has grown so much during the last two years that it now occupies a 14×14 planting space. The additional pods in this case mean only a slight additional efficiency. I.C.S. 3 was unfortunate in losing its cherelles time and again but succeeded in maturing about 50 pods. To date during the 1934-35 crop 20 ripe pods have been picked and 70 remain on the tree. I.C.S. 4 surpassed all expectations. It produced a total of 344 pods, but undoubtedly the pod size suffered as a result. Practically the whole of this was a branch crop and a number on the tips of the branches were small. However at least 200 of these pods must have averaged 7 to the pound which means that the tree produced cacao during the year at the rate of about two tons to the acre 60 per cent. of which consisted of superior beans.

I.C.S. 5 was robbed for budwood and cuttings but produced 69 fine pods which is its fair quota. I.C.S. 6 has grown considerably in size and now occupies a space of 16×16 feet. It matured 125 pods during the year. I.C.S. 8 suffered from excessive flooding and matured slightly less than 100 pods. I.C.S. 18 and 19 matured their quotas in spite of the poor average for the field in which they are situated. The former has a very heavy early crop in this coming season. I.C.S. 21 produced about 60 pods many of which were exceptionally fine and contained very large beans.

The outstanding performance of the year came from I.C.S. 22. It was mentioned in the last report that 79 pods had been reaped up to the end of 1933. The total crop amounted to 751 pods. This in itself is not outstanding, but assuming the pods averaged seven to the pound there were 22 lb. of superior beans produced at the rate of 30 cwts. to the acre while the field in which it was standing produced on the authority of the planter about 2 *cwts. per acre.* There was one other tree in this field which also bore well (I.C.S. 11) but apart from these two trees the yields of the remainder were negligible.

I.C.S. 100 produced 230 pods during the year which was well above its average of 191. Among these trees I.C.S. numbers 1, 4, 6, 18, 21, 22, 29 and 100 have been found to be largely self-compatible although some were rather late in setting crop. The remainder were not necessarily self-incompatible but were not tested since they did not come within the scope of pollination experiments.

Descriptions of further I.C.S. selected trees will now be given.

I.C.S. 7.

Situated in a loose gravel soil with a loamy covering this tree is younger than the average of the field. It occupies a space of 12×12 feet and has a characteristic dryish appearance of trunk and branches. This character has been noted for other trees of the same type. It is one of the completely self-compatible types and generally manages to set an early crop. The cherelles are very prone to wilting and this is attributed here to the dryness of the soil and the vegetative inertia of the tree under present conditions. The pod is warty with a slight bottle neck and partially pigmented on a white base. The pod is slightly larger than that of I.C.S. 2 but has almost as thin a shell. The average dimensions are 15×8.5 cms.

Beans number approximately 40 per pod and wet cacao 180 gms. The individual beans are thus well over the required standard.

For three years this tree has produced an average of 53 pods per annum but it is felt that its capacity would be much greater under better conditions. As it stands the yield is just up to one ton per acre.

I.C.S. 9.

This tree is situated about ¼ mile away from I.C.S. 7 and on exactly the same soil formation. It is quite a young tree, estimated at 15 to 20 years. As regards size it occupies a ground area of 14×14 feet and is of average height. Noted three years ago for the size of its beans it produced just under the required number of large pods for two adverse crop seasons and during the last year matured between 80 and 100 pods. It has not been tested for self-compatibility but this is undoubtedly its most valuable asset. The pod is long, thin and with rather a pronounced point. It is partially pigmented on a green base and warty. In dimensions the pods measure about 18×7.6 cms. and the shell is thin.

The bean number per pod is extremely variable. Well grown pods on the trunk and often on even the finer branches contain over 40 large beans but alongside such pods there may occur others with as few as 20 beans. In such cases the beans of the latter are very large and plump. This abnormally large variance of bean number may possibly be susceptible to correction by suitable manuring. Taking large and small pods together the average is about 34 per pod. Wet cacao averages 160 gms. which is equivalent to seven pods per pound of dry cacao. The individual beans weigh more than 4½ gms. and are thus well over the required size.

For the three years during which observations have been taken the crop has averaged 70 pods per annum which makes the yield equivalent to one ton per acre.

I.C.S. 10.

This is a large tree situated on a river alluvial flat often subjected to flooding. For a number of years it has been known to be a heavy bearer but not actually recorded. The pods are not large, they measure only about 16×7 cms. but the shell is almost as thin as that of I.C.S. 2 (Plate II. *3rd Ann. Rept.*). In colour the pods are partially pigmented on a white base. The surface is delicately warty.

The pods vary somewhat in size but the average is more than 40 beans per pod and the wet cacao 190 gms. The individual beans easily pass the size test. On the basis of six pods per pound of dry cacao and yields during the last two years of 150 pods per annum this tree passes our efficiency tests. If it were pruned smaller it would probably be much more efficient.

I.C.S. 11.

The origin of this tree is uncertain, it is definitely not of Trinidad. Like I.C.S. 10 it has been known for about ten years as bearing about 140 to 150 pods per annum. Situated on a comparatively fertile sandy alluvial site it covered an area of 18×18 feet until heavily pruned in 1934. Its reaction to pruning was to send forth after a brief vegetative interval, as many fruit as the branches would hold. For some time it has been tested and known as always self-compatible. Consequently whenever it produces flowers, new fruit set. At all times this tree seems to be holding fruit of all sizes and is one of the much valued continuous setters.

The pods are very dark red and very warty. There may be a suspicion of a bottle neck in some cases. There is a very characteristic red warty curved point. The bean number per pod is low averaging about 30 and although the individual beans weigh nearly 5 gms. each the pods work out at more than 7½ to the pound. The average is probably about eight and for this reason the description was withheld in the last report. However the beans are plump, light in colour and together with the tree's self-compatibility, the general efficiency cannot be far short of others yielding at the rate of more than one ton per acre.

I.C.S. 12.

The origin of this tree is interesting. It was one of the most vigorous seedlings from a pod of impure Nicaraguan Criollo cacao. The Forastero parent was not known but since the bean size is well above average and the beans themselves are light in colour there must be a good number of Nicaraguan genes inherited. The point of interest is the vigour of the tree which

far surpasses that of any pure Nicaraguan trees in Trinidad. Although only about 12 years old with the non-jorquetting habit of its maternal parent it occupies a space of 10×10 feet, is very robust and for three years has borne about 40 pods per annum. Its maternal parent seems to be extremely self-compatible but the daughter has off-periods.

The pods are long and green with no red pigmentation. They measure about 20×8 cms. There is a pronounced point, the surface is warty and the shell thin. The beans inside are very pale. The number per pod is low, in the region of 30, but the pods themselves average about 7½ to the pound since the beans are large and plump.

I.C.S. 13.

This and the succeeding two trees were recorded for one complete year when they were considerably damaged by the 1933 hurricane. However having passed the yield tests in the first year and shown signs of vigorous recovery since, they have been added to the list of selected trees for comparative tests.

Growing in a heavy clay soil on a slight slope this tree occupied a 12×12 feet space and produced between 50 and 60 pods in 1932-33 of which more than 30 were picked and weighed in one day. The tree is thus capable of nourishing many pods at a time.

The pods are long and unpigmented measuring about 22×11 cms. Prone to attacks of thrips in the present situation they usually appear russetted. The surface is warty with shallow furrows. The shell is about 1.3 cms. thick.

Beans average 40 per pod and wet cacao 180 gms. Thus the individual beans are considerably over the 4 gm. limit.

I.C.S. 14.

This tree is situated in an environment similar to the above. In 1932-33 it produced more than 70 pods and was then crippled by hurricane damage. It occupies a space of about 14×14 feet. The pods are short and oval, measuring about 14.5×9 cms. The shell averages 1.3 cms. in thickness. In colour the pods are partially pigmented on a green base. The surface is warty, the furrows are conspicuous but not deep.

Beans average 37 to the pod and wet cacao weighs about 154 gms. The individual beans are lozenge shaped.

At the moment the tree has much young growth and about 33 pods.

I.C.S. 15.

This tree stands slightly higher on the slope than the previous two. Before the hurricane it produced over 60 pods in the 1932-33 crop, over a space of little more than 12×12 feet. The pods measure 17.5×9.5 cms. with a shell 1.4 cms. thick. They are very heavily pigmented and slightly warty with a slight stalk end constriction.

The beans average 38 per pod and wet cacao 161 gms. Thus seven pods on the average give 1 lb. of dry cacao. The beans are plumper than those of I.C.S. 14.

There is much vegetative growth on the tree at present and a promising crop of 30 pods and many young fruit for 1934-35.

I.C.S. 16.

A tree situated on a heavy clay soil and occupying a space of 12×14 feet. The tree is rather low and spreading in habit. The pods are short and oval measuring about 16×9 cms. with a tolerably thin shell. They are partially pigmented on a green base. For two years the tree has produced more than 60 pods per annum at the rate of six to the pound of dry cacao or slightly less. The bean number per pod usually exceeds 40 and they are somewhat lozenge shaped. The yield of this tree has been about one ton to the acre each year.

I.C.S. 17.

This is a low branching budded tree whose parent was known to be good but the record was lost. It was subsequently identified with a seedling tree which had been used for budwood at the time this tree was propagated and which is still a very heavy bearer. At the time when buds were taken the old tree was producing 130 pods per annum but its size was not recorded and now only a chupon remains. The present pods give a rather low bean number but large individual beans. The budded plant gives a a variable crop. The 120 pods recorded since 1932 were composed of 95 in the first year and 25 in the second. During the present crop the yield promises to be exceedingly heavy.

The pods measure about 21×10 cms. with a shell 1.4 cms. thick. In the budded plant the bean number is high and the pods average 6½ to the pound of dry cacao, but the individual beans weigh 4.4 gms. as in the seedling parent. This may be an effect of stock.

The pods have a bottle neck but no point and are partially pigmented on a white base.

The low yield in the 1933-34 crop was partly due to severe pruning which took place early in 1933. Taken all round the tree promises to be up to the necessary standards of efficiency.

I.C.S. 20.

This tree is situated on a high free-draining gravel plateau. Its yield has been recorded as 70 pods per annum over a period of ten years. It occupies a space of 12×14 feet. and is a noticeable early bearer. Pods which are long and green are borne all up the trunk and not confined to the branches. They possess no red pigment. The shell is of average thickness and slightly warty.

Beans average about 43 per pod and are fairly plump. Wet cacao averages about 185 gms. Thus the individual beans weigh about 4.4 gms. each. Six and a quarter pods go to make 1 lb. of dry beans—the annual yield is about 11 lb. and the rate of yielding is in the region of one ton to the acre.

I.C.S. 24.

Growing on a well drained sandy alluvial site this tree occupies a space of 10×12 feet and is well branched and shapely in habit.

The pods are very pale green with no pigment present. They measure about 18×9 cms. The shell is of medium thickness and somewhat warty. There is no neck constriction or pronounced point, the pods being filled with beans to both ends.

Bean number averages 41 per pod and wet cacao 165 gms. Seven pods give 1 lb. of dry cacao and the beans are up to Superior standard.

During the 1932-33 crop the tree matured approximately 50 pods. In the succeeding crop the yield was less than 40 pods, but then practically all the trees in this field were poor too. During the present crop the total of picked plus nearly mature pods is 60 with a number of safe cherelles and the tree is still setting fruit. This makes up for the poor crop in 1933-34 and brings the yielding power of the tree to an average of more than one ton per acre per annum for the three years.

I.C.S. 25.

This tree grows in the same field as I.C.S. 24 and has been known as a producer of Superior beans for three years. Like I.C.S. 24 it produced a very good crop in 1932-33 but only a moderate crop in 1933-34. It commenced setting early in 1934-35 crop and by December, 1934, it was holding 58 mature pods, several smaller ones, and a good number of small sets.

The pods are somewhat similar to those of the preceding example but rather smaller. From an average of 50 pods, bean number was calculated at 34 per pod and wet cacao at 155 gms. Thus the individual beans are Superior in size and the tree averages about one ton per acre.

I.C.S. 26.

This tree comes from the sandy soils of the Northern Range. It is growing on a very steep slope and consists of several chupons. Known for several years as a heavy bearer of about 100 pods per annum it appears to be one of the continuously self-compatible trees. The pods are oval in shape with rather a pronounced point. They are rather heavily pigmented on a green base and average about 20 cms. in length. The beans are plump with about 37 per pod and 170 gms. of wet cacao. The individual beans weigh about 4.6 gms. each.

The space occupied is about 15×15 feet and from the above figures of 100 pods per annum at 6⅔ pods to the pound the yield is well over one ton to the acre. The crop in 1934-35 will be rather above 100 pods.

I.C.S. 27.

A tree situated in a good sandy loam soil, not subject to flooding, and of good average fertility. It occupies a space of a little more than 12×12 feet and was heavily pruned in 1934.

The pods are of average size, about 17×8 cms. and the shell is rather thinner than normal. The surface is warty with rather pronounced furrows. There is no long point or neck constriction. The pods are white with splashes of red pigment.

Bean number averages well over 40 per pod and wet cacao about 200 gms. Thus about six pods should give 1 lb. of dry cacao, and the beans are well over standard.

The crop was heavy in 1932-33, poorer in 1933-34 and promises to be very heavy in 1934-35. To date 60 pods have been accounted for and the tree is still setting more.

I.C.S. 28.

Also growing in a sandy loam soil this tree has been noted as a heavy bearer for about ten years and used for supplies. The important feature is the thin pod shell. The tree was very large up to 1934 when it was pruned back heavily. In its old state it had been occupying a space of about 18×18 feet and bearing about 150 pods per annum but in 1933-34 the yield fell off. After pruning it set up a very heavy crop again for the 1934-35 season. The tree has many Criollo genes present judging by the pod characters of thin shell, wartiness and an appreciable point. It is green and unpigmented.

Beans average 40 per pod and wet cacao 180 gms. The individual beans are large and the yield must be well over one ton per acre per annum.

I.C.S. 33.

This is a tree which is only 12 years old growing on a very fertile steep slope of a hillside. It occupies a full 14×14 feet spacing distance but is not unduly large. For several years the tree has been a noted heavy bearer—on the authority of the owner, since it first came into bearing. It has not been actually recorded until this season when to the end of December 115 pods had been accounted for.

The pods are unpigmented on a white base rather smooth and larger than normal. There is no bottle neck constriction or long point. Bean number appears to be about 40 and wet cacao 200 gms. per pod. On this basis the yield cannot be less than at the rate of 30 cwts. per acre.

I.C.S. 97.

This tree grows on the side of a ravine on a high gravel plateau. There is some alluvial deposit above the gravel. The tree consists of five long chupons which bear pods both on the trunks and in profusion in the branches. Altogether the tree occupies a space of 20×20 feet and has produced an average of slightly more than 200 pods for two consecutive years. The pods are long and oval with just a suspicion of bottle neck but no curved point. The surface is green, unpigmented and warty. The mean dimensions are approximately 20×9 cms. and the shell is thinner than normal.

Beans average 38 to the pod and wet cacao 140-150 gms. Thus both beans and pods are slightly below first class standards but the yield is well over one ton per acre. Some budded plants of this tree are bearing heavily even in a soil very deficient in potash.

Descriptions have now been given of 36 trees out of 100 which will finally be selected. Altogether about 250,000 trees have been surveyed and some 250 are on the provisional lists. It is hoped that by the end of the 1934-35 crop the number found which yield at the rate of at least one ton to the acre will exceed 100 and final selections will be concluded. At the present all on the I.C.S. and provisional lists are being tested for resistance to Witches' Broom disease, as there is just a possibility that some may prove resistant. A number of planters have kindly offered to plant seedling progeny raised by hand crossing between various selected trees, so that in a few years time, still more favourable combinations of genetic characters may be obtained.

CERTAIN BARREN TYPES OF CACAO.

By F. J. Pound.

The study of an extremely heterogeneous plant population usually reveals the presence of a number of types which are of no commercial value whatever, and the cacao tree population is no exception to the rule.

Under this heading comes the so-called " Male tree ". This name is given not to emphasize the value of such trees as pollinisers but because they produce little or no fruit and they have been described here to prevent any confusion with the ' male ' trees, which in other crops may be useful pollinisers.

Male trees in cacao are of two kinds.

(1) The incompatible tree, which has potentialities of fruit formation in that the essential organs are complete, and

(2) the male and female sterile tree which has no potentialities of fruit formation on account of structurally sterile organs.

In addition there occur trees which produce masses of small pods the beans of which cannot be extracted without difficulty and if extracted are of no commercial value. These three classes will be dealt with in turn to illustrate their worthlessness in the cacao field.

1. The Incompatible Tree.

The problem of incompatibility has been fully discussed elsewhere, and whether or not truly self-incompatible trees exist does not here concern us. Certainly there are many which set very little fruit and for all practical purposes are self-incompatible. These trees are usually prolific flowerers. It is undoubtedly a fact that many cacao trees are shy bearers from youth owing to pollen incompatibility but it is becoming evident that this condition is aggravated by age in some trees. These types can often be rejuvenated by severe pruning which causes the formation of fresh vigorous wood and probably more efficiently functioning roots. The reason why in some cacao districts one hears that normal trees are becoming male may be due to this age tendency. The age effect, however, depends for its expression largely on the individual tree since some old trees exist which are still efficient producers of cacao.

The flowers on these incompatible trees are often not only self-incompatible but their pollen will not cause setting on some self-compatible neighbours even though it may be viable on a five per cent. sucrose medium. They can thus be of no importance as ' male ' trees since in cacao we have already demonstrated that the necessity for foreign pollen is only felt with self-incompatible trees and then only pollen from a self-compatible tree is effective. This is the first class of tree which should be replaced by a more efficient type.

2. The Sterile Tree.

This class of tree, known also as ' Male ' is actually female sterile and usually male sterile as well. There can be no doubt of the inefficiency of such types. In the majority of cases they set fruit in profusion but then no trace of ovules can be found, whence it appears that developing seeds are not essential for ovary growth in these types. It is not clear yet whether pollen is necessary, for from numerous hand pollinations with pollen from strongly self-compatible trees no sets have been registered. These trees like normal ones may refuse to set except at certain periods, but here during the off-periods even self-compatible pollen is ineffective. The pseudo-cherelles grow to varying sizes depending on the genetic constitution of the tree. The majority ripen on reaching a diameter of $\frac{3}{4}$-inch or less. Others grow larger. No beans have been found inside these fruit. Dissection of the flowers shows that ovules are normally absent but if they occur they are few in number and often massed at the base of the ovary. Since such ovule structures are sometimes present they may be necessary for setting but they never develop into seeds.

The floral morphology of some of these trees is interesting if only to point out to the cacao planter the complete uselessness of the type.

From several trees examined there is no constancy in the morphology of the pistil. Non-vital floral members are normal. Stamens are normal in appearance or partially divided (Fig. 1), usually development is normal to about the stage of pollen mother cells when breakdown

FIG. 1.—*Stamen of 'Male Tree'. The pollen sacs contain brownish disorganised cells.*

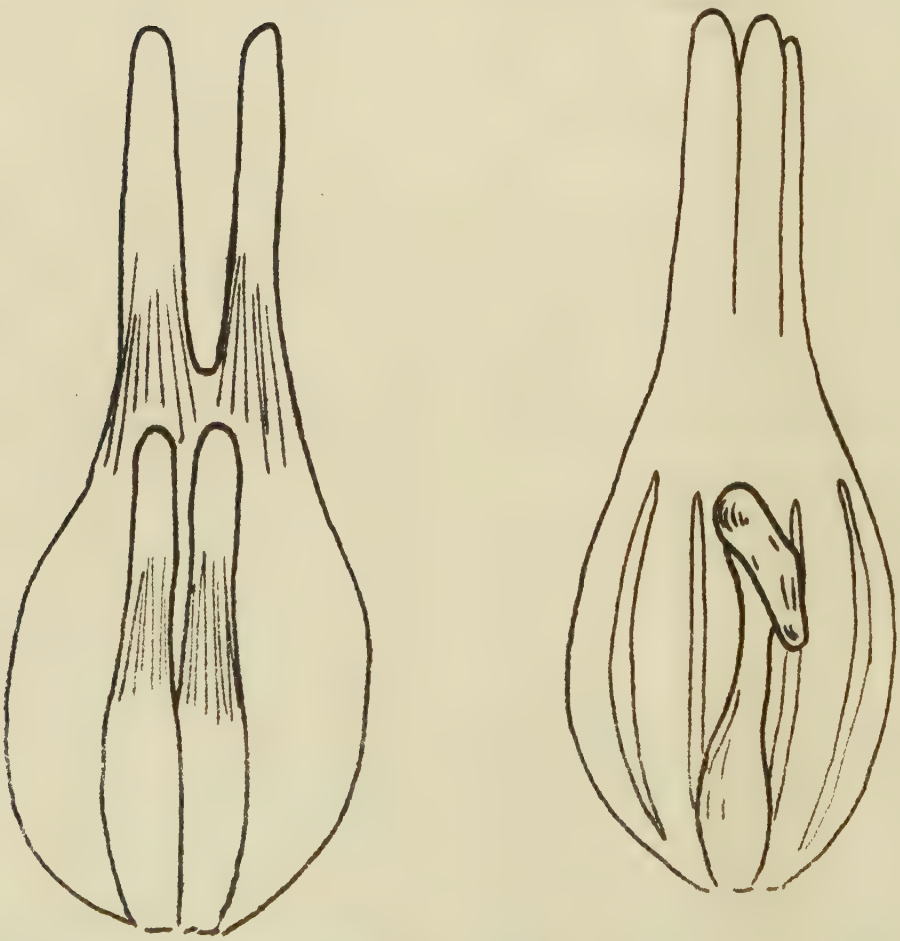

FIG. 2.—*Dissections of ovaries showing the remaining stigmas becoming pigmented and parenchymatous structures arising from the base of the ovary.*

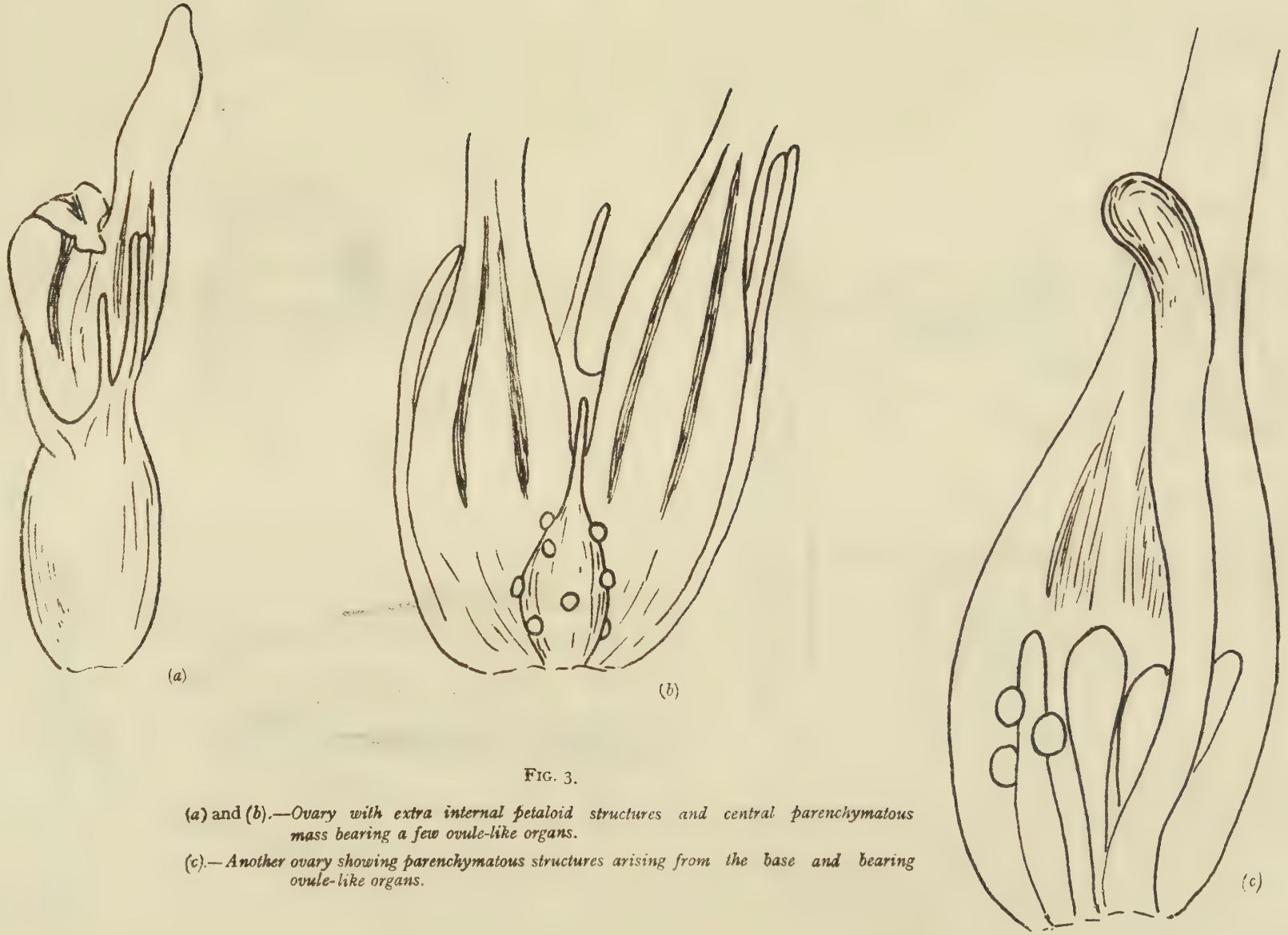

FIG. 3.

(*a*) and (*b*).—*Ovary with extra internal petaloid structures and central parenchymatous mass bearing a few ovule-like organs.*

(*c*).—*Another ovary showing parenchymatous structures arising from the base and bearing ovule-like organs.*

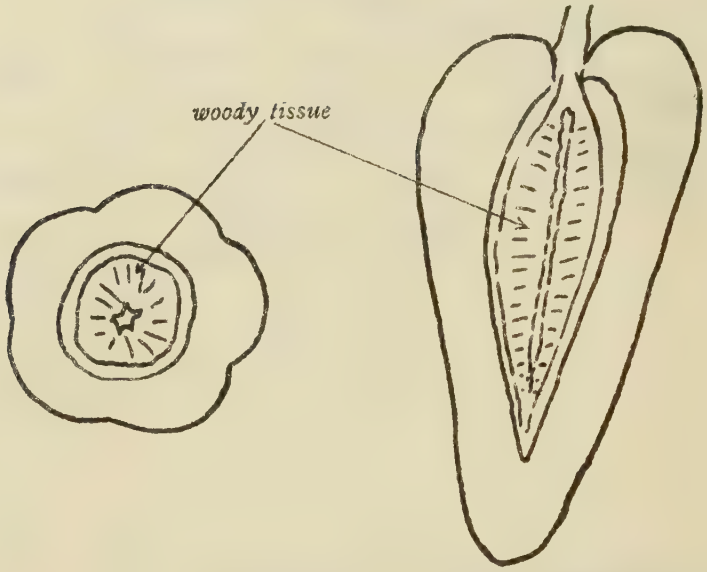

FIG. 4.—*Pseudo-cherelle with woody internal tissue extending practically to the centre.*

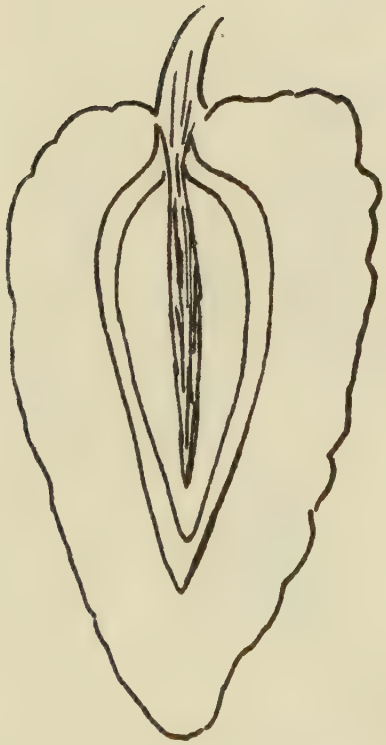

FIG. 5.—*Pseudo-cherelle with central woody core.*

FIG. 6.—*Half grown pseudo-cherelle with mass of glistening cells at base of ovary.*

occurs and no pollen grains are formed. In place of pollen sacs there then appears a mass of brownish cells. Some ovary types are curious. Fig. 2 illustrates a common kind where the few remaining stigmas are becoming staminodal in appearance, and the ovary consists of wall only on the inside of which are purple stripes. In the majority of cases in addition to the pigment in the ovary wall parenchymatous structures rise from the base of the ovary or even from the wall itself, which are pigmented at the apex like the modified stigmas (Fig. 3c).

Usually these structures, which appear to be placental in origin, are completely barren, but ovule structures may occur as in Fig. 3b and c. As far as can be ascertained there is no embryo sac inside these ovule-like organs.

When the ovary grows to maturity it may become hard and woody and difficult to cut. (Fig. 4), shows how abnormal woody tissues may develop around a central mass of parenchyma. Sometimes a woody core occurs in the very centre of the fruit (Fig. 5). Very occasionally groups of glistening cells looking like ovules, persist at the base of the cherelle until the fruit is about half grown (Fig. 6).

3. The Small Podded Tree.

Usually these trees are self-compatible and heavy croppers. The bean number is normal or rather higher than normal. It is common in these types to find no break-down of parenchymatous tissue around the beans to form the usual mucilaginous covering. The seeds if they can be extracted, are small and dry. In many cases examined the beans fail to separate from one another and the pod wall and often they are non-viable. Such types have been known to appear among seedling progeny of tolerably good types. Their origin is not known, but it is probable that they are extra-parental segregants formed by abnormal combination of chromosomes. Usually the cotyledons are purple but not always. The beans are always small, requiring commonly more than 1,000 to the pound instead of the usual 400, and the pods range from 20 to 40 to the pound of dry cacao. Even supposing that such trees could produce several hundreds of pods on a 12×12 feet planting space they would be uneconomical. 30 pods have to be picked and broken for each pound of dry cacao and the final product serves only to detract from the commercial value of the better cacao with which it is mixed. There should be no hesitation about removing every tree of this nature from the plantation. If they function as pollen parents the effect is bound to be detrimental on the next generation to say nothing of the quality of the beans in the present.

Summary.

Certain barren types of cacao tree are described to illustrate their uselessness in the cacao field.

A NOTE ON A METHOD OF CONTROLLED POLLINATION OF CACAO.

By F. J. Pound.

In the 10th Annual Bulletin of the Department of Agriculture, Nigeria, Voelcker describes a simple method of isolating a cacao flower for controlled pollination. The whole apparatus consists essentially of a matchbox cover, a piece of fine gauze cloth and a length of tape, the gauze cloth covering one open end of the box while the tape serves to hold the apparatus on the branch. Voelcker finds this apparatus quite satisfactory over a nine month trial although he does not state the percentage of successful pollinations obtained. Of 59 controlled flowers not pollinated none set fruit which proves to his satisfaction that the apparatus is probably sound in principle.

At the Imperial College controlled pollinations have now been carried out for a period of a little over four years. The form of apparatus used in isolating the cacao flower is very similar to that used by Voelcker but it probably has certain advantages. Instead of the matchbox which shuts off practically all the light which may be essential to the flower a glass tube of 1-inch diameter is used and fine gauze cloth is fastened to one open end as in the case of the matchbox. The apparatus is fixed to the branch simply by a small quantity of plasticine which removes the necessity of tape and tying and makes a perfectly insect proof joint (Plate II. Fig 4). The method of fixing is to roll a piece of plasticine into a cylinder 3 to 3½ inches long by ½-inch diameter. This is fixed around the cushion containing the buds, to form a ring and pressed to the bark in order to fill crevices and fissures. The open end of the glass tube is then pressed on to the ring. Before using plasticine as an adhesive the writer had experimented with tape but owing to the varying lengths required to meet branch diameters varying from one inch to 12 inches the method was discontinued.

Further under Trinidad conditions grease and a green baize cloth failed to prevent ants from entering the box and possibly effecting pollination, owing to the irregularities of cushion growth and bark fissures.

The method of pollination was similar to that described by Voelcker, mature buds were covered on the afternoon before opening and pollinated on the following morning. Never less than two and usually four or five anthers were lightly brushed across the stigma *until it could be clearly verified by the unaided eye that ample pollen had been transferred.* This was necessary because

in some cases anthers brought from uncontrolled flowers contained very little or no pollen owing to the depredations of thrips and other crawling insects.

In all the tests carried out the writer is not certain of any natural set taking place within the apparatus without the presence of insects. Whether or not this proves the necessity of insect visitation cannot definitely be stated since even buds would occasionally absciss before opening and flowers certainly abscissed earlier than normally. From other experiments described in the First Annual Report (1931) time of flower abscission would appear to be a limiting factor in fruit setting. For this reason it was usual on pollinated flowers to remove the apparatus 24 hours after pollination since Cheesman states that actual fertilisation takes place within a few hours of pollination.

In view of the inevitability of interference with the natural environment of the flower, in any attempt at controlling pollination by apparatus, it is worth considering the extent to which such apparatus must be regarded as necessary.

Observations on trees in Trinidad show that as long as a tree will not set fruit on self-pollination under controlled conditions it also sets none naturally from unguarded flowers, with very few exceptions. Since in such cases pollen brought from a neighbouring tree is usually capable of setting fruit it appears evident that there can be little natural transport of such pollen. The highest figure recorded for pollination between grease bands is 3.8 per cent. of flowers (Stahel) and this includes possible transference of pollen by flying insects from flowers of the same tree.

If we consider the figures of Harland and Stahel that five to six per cent. of flowers are pollinated by natural means and also the work of Harland showing that about 30 per cent. of *fruits* are cross-fertilized we conclude that at most only 1.5 to 2.0 per cent. of *flowers* can be cross pollinated. This is in line with our observations and there are many purposes for which such a figure can legitimately be regarded as negligible. Furthermore there are good reasons for supposing that so high a proportion of natural crossing as 30 per cent. can only occur on self-incompatible trees.

On self-compatible trees in studying ovule loss from genetic causes a comparison has been made between selfings and crossings performed without any guard or emasculation, and the results are so clear-cut as to show quite definitely that the risk of contamination with self-pollen is negligible. In this case however care is always taken to see that the stigmas are perfectly free of pollen before the cross is made.

Pollinations for the raising of hybrids between a tree which is in the self-compatible state and one in the self-incompatible state can be performed with little danger of interference if the self-incompatible tree is used as female parent, for in this case self pollen is ineffective.

In a few cases, ample sometimes for the natural sets on self-incompatible trees, pollen has been found present on the stigmas soon after the flowers opened. In the several cases examined the flowers were free of insects and as usual there were no insects flying around the trees. Occasionally it was noticed that when a flower opened on a fine dry morning the anther was liable to explode and pollen would be shot out of the hood of the petal. In most cases this pollen landed on the staminodes but when a flower is hanging at an angle of 45° to the vertical it is possible for the pollen from one anther to strike the stigmas. Very little importance can at present be attached to this phenomenon.

The fact remains that for experimental work it may still be advisable to cover the bud in cacao when critical crossings or selfings are required but for commercial purposes and fruit setting studies there is probably no advantage to be gained considering the circumstances of self-incompatibility and the abnormal environmental conditions produced.

Summary.

A simpler method of isolating cacao flowers for controlled pollination is described.

A review of pollination studies suggests that only in very critical cases is the precaution necessary.

STUDIES OF FRUITFULNESS IN CACAO.

V. Conditional Self-Compatibility and its Implications.

By F. J. Pound.

In the second paper of this series it was reported that of a number of young budded clones some would set fruit readily with their own pollen whilst others set little or none even after trials extending over several months. This proved that the value of self pollen in setting may vary from clone to clone under similar environmental conditions.

The third paper of the series added the information that a number of comparatively young seedling trees, uniform with respect to age and environment, could be classified as self-compatible or self-incompatible. The former always set fruit from hand selfings over a period from May to October irrespective of the number of flowers on the tree and of the state of the flush. The latter failed to set more than ten per cent., and remained self-incompatible throughout the whole period. It was found that pollen from a self-compatible tree was effective on any stigma, but pollen from a self-incompatible would only cause setting on stigmas of a self-compatible. No case was noted where reasonable setting followed the crossing of two self-incompatibles, although earlier observations had suggested the possibility of such an occurrence (*1st Ann. Rept.* p. 27. Cross 2×6).

These observations were supplemented by Marshall (*3rd Ann. Rept.* p. 34) who carried out similar experiments on another block of young trees at River Estate. Over a period from October to May, trees retained their relative positions as self-compatible, self-incompatible, or partially self-compatible without change. Thus it is clearly established that there is some genetic foundation for the ability to set, comparable with that found by other workers in temperate orchard crops.

Clear cut as the cited results appeared, however, they left open for further explanation several important facts. It was difficult, for example, to understand why among trees self-compatible at one time, and therefore presumably self-compatible at all times, some should be early bearers and some late. It was also difficult to explain how, whilst the efficiency of insect pollinators on observation plots of self-incompatible trees was obviously very low, certain trees classed as self-incompatible could nevertheless set up a fair crop. In the course of this year's work *conditional* self-compatibility has been discovered, and its occurrence throws much light upon these outstanding problems. It now appears that cacao trees can vary in their condition, so that a tree self-compatible at some times may be the reverse at others.

In the summer of 1934 attempts were made to cross certain trees indicated by the selection programme as likely parents of particularly desirable progeny. Since these were all comparatively heavy bearers, no pollen incompatibility was expected to occur. It was found, however, at the commencement of the work in May, that sets were only registered in certain cases, and it was in fact proved that at that time only two out of nine trees used at River Estate and only two out of six at La Vega were in the self-compatible condition. The remaining trees were flowering profusely but setting no fruit either naturally or from crosses between them. They would, however, set with pollen from the self-compatibles, and their own pollen was effective on the latter. Eleven out of fifteen selected heavy bearers were thus behaving in May, 1934, in all respects as self-incompatible trees. There was no visible reason why they should differ in behaviour from the other four, and their behaviour must lead to one of two assumptions. Either these heavy bearers normally set all their fruit from cross-pollination, or else they were only temporarily self-incompatible. The first assumption seemed most improbable in view of the general inefficiency of cross-pollinating agents, and steps were taken to check the second by hand pollinations made from time to time.

Observations were carried on through what subsequently proved to be a highly abnormal season, since the rains that usually begin in May were delayed until September. From the point of view of the investigation in hand, this was perhaps fortunate. In normal years a great burst of setting takes place soon after the advent of the rains, indicating that, as might be expected, soil moisture is one important factor in setting. The behaviour of the four trees that were proved self-compatible and were setting fruit naturally at the end of May is sufficient to show that soil moisture is by no means the only factor involved and the postponement of the major environmental change from dry season to wet season conditions served further to confirm that point, as will appear.

At the beginning of June several of the eleven self-incompatible trees came very near to setting. Abscission of the flowers was delayed sometimes for several days particularly in the cases of I.C.S. 1 and I.C.S. 4. During June conditions were so arid that most of the trees ceased flowering and even the self-compatible trees registered poor setting. In July conditions were more humid but no definite wet season had commenced. However, at the beginning of the month I.C.S. 1 recommenced flowering and started setting fruit naturally as

well as from hand selfings. This condition was kept up till the end of the year. Here then was the first instance of a cacao tree changing its condition as regards pollen compatibility and coinciding with this change there occurred a general upward trend in setting over observation plots. As expected, pollen from I.C.S. 1 was now found to be capable of causing setting, although not prolific in some cases, on the remaining incompatible trees.

Early in August I.C.S. 4 and I.C.S. 29 both changed over to a state of self-compatibility but after a short period of intense setting the latter ceased flowering altogether. From this time onwards sets between the remaining self-incompatible trees became more frequent and natural setting commenced to some extent on most. I.C.S. 18 and I.C.S. 19 both at La Vega put on heavy crops of young fruit and ceased flowering. At the end of August only three of the trees at River Estate and one at La Vega could definitely be classed as self-incompatible. These continued flowering more or less profusely and began to take on crop during November and December, not in a sudden burst as the others had done but in occasional sets all over the tree. At the same time occasional hand selfings were successful.

The evidence from these trees is enough to prove that self-compatibility in cacao, though fundamentally genetic, depends very much on other factors for its expression. It is interesting to compare this behaviour of a tropical crop capable of producing flowers at almost all times, with that of temperate crops of which the flowering period is usually narrowly restricted. In June and July (the months of maximum setting in normal years) cacao trees in Trinidad could be classified into self-compatible and self-incompatible with all the attendant genetical theories, as definitely as can cherry, plum or apple trees in England. Yet, a tree self-incompatible at this period of general setting may become self-compatible at some other time, and being still in flower may set up a heavy crop. Pending further knowledge one is forced to doubt whether a truly self-incompatible cacao tree, that is to say one genetically incapable of self-compatibility, exists. The doubt does not, however, contradict the view that some trees, by virtue of genetic constitution, are more constantly self-compatible than others.

Conditional self-compatibility serves to explain how a tree may be an early bearer in one season and a late bearer in the next, or may fail for a whole season to set any crop except for a few pods. Also it changes the angle from which problems of fruit setting are to be viewed. Heavy setting and a self-compatible condition were previously noted as going together, and the conclusion was drawn that " much more setting might take place on self-incompatible trees at certain times if there were more efficient agents of cross-pollination in the cacao field " (*2nd Ann. Rept.* p. 36). Now that the incidence of setting on trees previously self-incompatible has been observed to coincide with a change to self-compatibility, it is no longer necessary to postulate even occasional abundance of crossing agents in explanation of crop on such trees, and in the absence of any direct evidence for its occurrence, any large amount of natural crossing at any time must be considered doubtful. The evidence now suggests that the bulk of the Trinidad cacao crop is produced as a result of self-pollination on all trees, and further that under conditions favouring self-compatibility the amount of setting will largely take care of itself, at least on trees of good genotype. The problem of increasing yield thus becomes a question not so much of increasing the intensity of setting during certain limited periods as of prolonging the duration of periods over which the tree is self-compatible.

In this connection it is interesting to recall an observation of Pyke (*2nd Ann. Rept.* p. 39) that of 38 comparable trees at River Estate in the 1931 crop, 24 were " continuous setters " and gave an average yield of 80.4 pods per tree, whilst 14 were " intermittent setters " and averaged only 37.6 pods per tree. There can now be little doubt that the " continuous setters " were the trees that were also continuously self-compatible. The connection between continuous setting and high yield is not far to seek. Once a young crop has been set, its survival through the critical stage of greatest susceptibility to shrivelling appears to be largely a matter of chance, depending on weather conditions over a relatively short period. When the set has been heavy, the drain on the tree at the critical stage is correspondingly severe, and the whole of the cherelles may wilt under conditions that would have permitted a lighter set to survive. If on recovery the tree is no longer self-compatible, the crop is lost for the season, but on a continuously self-compatible tree fresh fruit may be set which this time may survive. Thus so long as the self-compatible condition can be maintained the crop can be spread over a longer period and the effects of short spells of adverse conditions must be much less disastrous.

There is evidence to show that mineral nutrition is among the factors to be considered. By application of dressings of phosphate to some Trinidad soils, and potash to some others, the Chemical Section has demonstrated large increases in yield. It becomes of importance to determine whether increased intensity of setting or prolongation of setting period is mainly involved. Detailed observations are in progress on the manurial experiment at River Estate described in the fourth paper of this series. Data are withheld until they can be analysed for more than one major setting period, but for the first year they suggest both effects in the case of phosphate and prolongation in the case of potash on this site.

Summary.

Evidence has been obtained that cacao trees self-incompatible at one time may become self-compatible at another. Physiological factors are thus proved to modify the expression of genetic constitution with respect to self-compatibility. Continuous self-compatibility is favourable to high yield in cacao, and consequently any factors tending to prolong the self-compatible periods of " intermittent setters " will tend to increase yield. It is from this point of view that setting problems must henceforth be investigated, and the qualitative effects of mineral nutrition are already under observation.

STUDIES OF FRUITFULNESS IN CACAO.

VI.—First Year Observations in an Experiment Designed to Test the Gross Effects of Applications of Nitrogen, Potassium and Phosphorus on the Cacao Tree.

By F. J. Pound and J. de Verteuil.

It is not intended in this paper to discuss the details of this experiment since an adequate description has already been published in the fourth paper of the series. Sufficient is it to explain that the experiment is being conducted jointly by the Cacao Research Officers and the Trinidad Department of Agriculture and takes the form of a complete manurial trial on established cacao, relatively uniform for age and in full bearing. The site is a normal flat alluvial field at River Estate. The design is a 4×4 Latin Square to test the effects of nitrogen and potash upon which is superimposed a half-plot treatment of phosphate. Each main plot consists of 16 seedling trees in two rows of eight. The half plots are eight trees in two rows of four. For other details the reader is referred to the description in the 3rd Annual Report.

Of the points examined the following come within the scope of this paper :—

(1) The suitability of plot size and design.
(2) The relative values of variance due to (*a*) Soil heterogeneity and (*b*) ' Error '.
(3) The value of natural yields collected previous to the experiment for increasing precision
(4) The effect of treatments on the bearing capacity of the trees.
(5) The effect on Bean Weight.
(6) The effect on Bean Number.
(7) The effect on the incidence of Black Pod.
(8) The effect on Fruit Setting.
(9) The effect on premature loss of young fruit.

The Analysis of Pod Number.

This is not in itself a final analysis of yield owing to the variance of pod value from tree to tree but it serves as an indication of bearing capacity. In the analyses below, diseased pods which had almost reached maturity have been counted as mature, since the trees had completed their work of nourishing these pods even though no beans of commercial value were obtained from them. For two years previous to the application of manures, records of pod yields had been taken from each tree in order to demonstrate how far such records are useful in increasing the precision of judging the effects of the manures in the experiment years.

For convenience the yield records of these years together with their analyses of variance, are repeated below. For discussion of the variance in the previous record years the reader is referred to the earlier report. Tables 1 and 2 give the yields arranged in half plots.

Table 1.

Natural Yields, 1931-32.

Mean Number of Pods per Tree.

28	28	26	22	14	54	55	26
40	18	21	31	31	31	37	30
28	22	35	30	33	25	33	33
26	26	34	19	50	41	59	37

Table 2.

Natural Yields, 1932-33.

Mean Number of Pods per Tree.

34	29	31	22	5	38	36	26
41	24	11	20	23	28	32	30
17	14	26	17	22	31	19	19
19	17	6	8	40	33	27	14

The Analyses of Variance with hypothetical treatments are shown in Tables 3 and 4.

The Analyses of Variance for the two years show that by accident of design there appeared an unusually large variance of interaction of nitrogen with phosphate. Hence if this appears significant in the experiment year the result will be open to doubt. Otherwise no ' treatment ' approaches significance and the design is satisfactory. Thus without actually using co-variance on experimental yields previous records have their value in indicating possible flaws in the interpretation of manurial results.

Table 3.

Analysis of Variance—Natural Yields, 1931-32.

Due to	D.F.	Sum of Squares.	Variance.
Rows } Soil	3	235.38	78.46
Columns } heterogeneity	3	811.13	270.38
Plot Treatments	3	26.63	8.88
Nitrogen	1	3.81	3.81
Potash	1	22.81	22.81
N × K	1	0.01 } 463.37	0.01 } 66.20
Main plot Error	6	463.36 }	77.23 }
Main plot Total	15	1,536.5	....
Total Treatments	7	388.25	....
Sub-plot treatment	1	34.06	34.06
Inter. SP × MP Treatments	3	327.56	....
N × P	1	205.01	205.01
K × P	1	108.76	108.76
N × K × P	1	13.79	13.79
Sub-plot Error	12	1,640.85	136.74
Grand Total	31	3,538.97	....

Table 4.

Analysis of Variance—Natural Yields, 1932-33.

Due to	D.F.	Sum of Squares.	Variance.
Rows } Soil ...	3	327.85	109.28
Columns } heterogeneity	3	436.85	145.62
Main plot Treatments	3	221.10	73.70
Nitrogen	1	11.28	11.28
Potash	1	175.78	175.78
N × K	1	34.04 } 879.21	34.04 } 125.60
Main plot Error	6	845.17 }	140.86 }
Main plot Total	15	1,830.97	....
Total Treatments	7	611.22	....
Sub-plot Treatment	1	42.78	42.78
Inter. SP × MP Treatments ..	3	347.34	....
N × P	1	247.54	247.54
K × P	1	94.54	94.54
N × K × P	1	5.26	5.26
Sub-plot Error	12	655.38	54.62
Grand Total	31	2,876.47	

For the first experimental year the pod records are given in Table 5 and the Analysis of Variance in Table 6.

Table 5.

Mean Pod Yields per Tree, 1933-34.

37	30	25	27	13	42	48	32
49	23	14	27	37	31	37	37
22	17	13	20	24	26	40	28
11	14	20	13	36	55	66	45

Table 6.

Analysis of Variance—Pod Yields, 1933-34.

Due to	D.F.	Sum of Squares.	Variance.
Rows	3	415.13	138.38
Columns	3	2,144.38	714.79
Main Plot Treatments	3	745.38	248.46
Nitrogen....	1	81.30	81.30
Potash	1	657.10	657.10
N × K	1	6.98	6.98
Main Plot Error	6	771.61	128.60
Main Plot Total	15	4,076.5	—
Total Treatments	7	1,143.25	—
Sub-plot Treatment	1	34.1	34.1
Inter. Sp. × MP Treatments	3	363.77	—
N × P	1	74.98	74.98
K × P	1	257.55	257.55
N × K × P	1	31.24	31.24
Sub-plot Error	12	1,158.63	96.55
Grand Total	31		

Clearly of the main plot treatments only applications of potash appear beneficial. 'Z' calculated amounts to 0.88815. For a significance of 20 : 1 this must exceed 0.8606. Hence the result would occur by chance less than once in twenty trials and may be considered significant.

The interaction of potash and phosphate is large and positive but not quite significant. Rows and columns together account for half the total variance showing that there is probably a natural potash gradient across the block.

The potash treated plots produced 4388 pods compared with 3241 from non-potash plots. The increase is of the order of 30 per cent. and the results fit in with expectation from previous work on uniformity trials (5).

(5) Cheesman, E. E. and Pound, F. J.—*Trop. Agriculture*, 9 (1932) 277.

The Value of Previous Records.

It was demonstrated by Cheesman and Pound (*loc. cit.*) that natural yield records may be very useful in correcting yields in experimental years and thereby increasing the precision and value of the results. The number of years over which such records should be taken for best results varied but the writers came to the conclusion that two years was probably the most usually effective period. The use of regression in this way with experiments on perennial heterozygous crops cancels a large amount of the difference in capacity for yield which different varieties possess, whether this is due to age or genetic constitution.

For economy of space tables demonstrating the Analyses of Co-Variance of the 1st Experimental year with each of the two preceding years have been omitted. Table 7 shows the relative precision which may be obtained by use of regression.

Table 7.

Table of Relative Precision.

Main Plot Units.

Year.	Variance.	Co-Variance with 1933-34.	Vy. r.	Rel. Precision.
1933-34	111.23	—	111.23	1.00
1932-33	125.60	58.92	97.52	1.14
1931-32	66.20	76.23	27.36	4.07
Sub-plot Units.				
1933-34	96.55	—	96.55	1.00
1932-33	54.62	50.78	53.82	1.79
1931-32	136.74	89.12	41.97	2.30

From a knowledge of conditions obtaining during the crop year 1932-33 it was not expected that regression using this year would be of very great value. Crops in all districts were abnormally low owing to adverse weather. Actually weather conditions in the first experiment year were little better and since in spite of this, potash shows beneficial effects, the results are all the more valuable.

Using the yield records of 1931-32 to correct those of 1933-34 the regression factor is calculated as 1.15 for Main plot treatments and 0.652 for Sub-plot figures.

The Analysis of Variance so corrected is given in Table 8 where for simplicity only the values for treatments are shown.

This table presents quite a different appearance from that of Table 6. Potash is still the only treatment which is generally significant. Nitrogen depresses yields by a very nearly significant

amount. The effect of phosphate instead of being very subnormal is high, positive and nearly significant. The same applies to the interaction of potash and phosphate which suggests that on this area phosphate is only beneficial when the potash deficiency has been made up. There is now no interaction between nitrogen and phosphate.

Table 8.

Analysis of Variance of (y-bx).

Due to	D.F.	Sum of Squares.		Variance.
Main plot				
Treatments	3	540.593		180.20
Nitrogen	1	126.658		126.658
Potash	1	405.861		405.861 (− 0.874)*
N × K	1	8.074		
Main plot			164.163	27.360
Error	5	156.089		
Phosphate	1	92.951		92.951
N × P	1	0.374		0.374
K × P	1	84.988		84.988
N×K×P	1	10.578		10.578
Sub-plot				
Error	11	461.519		41.956

Since the co-variance is large and positive an approximate picture of the effect of manures can be given by the simple differences in yields in the two years. These are shown in Table 9.

Table 9.

Yields of 1933-34 minus those of 1931-32.

	NK	K	N	C	
+P	+16	+22	−23	−14	+ 1
−P	−14	+ 3	−42	−12	−65
	+ 2	+25	−65	−26	

This demonstrates that when applied alone phosphate is of no particular value, but when applied in conjunction with either potash or nitrogen it is beneficial—in the case of nitrogen, phosphate reduces the detrimental effect.

Soil analyses made by the Chemical Section prior to the commencement of the experiment demonstrated that compared with normal standards both phosphate and potash were deficient. The phosphate status was equal to that of the other poor bearing sites but the potash deficiency was much more marked. Thus it was anticipated that potash on its own would be beneficial and that phosphate would also be beneficial in aiding the trees to absorb more potash than usual from the soil. Actually the site seems to be so deficient in potash that this effect of phosphate, well marked in other localities according to McDonald's experiments, does not appear and it is not until the potash status has been improved that phosphate is beneficial, and then it is of great benefit. Nitrogen appears to exaggerate the potash deficiency in the yield figures, a result in common with other experiments on temperate orchard crops, and it is interesting to note the extent to which phosphate counteracts this effect.

The actual effect of added nitrogen may be more obscure than appears from these figures. Evidence is to hand that nitrogen is of value for setting at certain periods although in total returns it is detrimental to crop. There is also evidence that nitrogen improves vegetative growth and tree canopy, which cannot in the long run fail to have a beneficial effect on yield.

To summarise, quite erroneous results may be obtained without the use of natural yield records, from fertilizer trials on established cacao. Definite increases in yield can be demonstrated by experiments of this type following the first application of manures. In the particular type of soil found at River Estate where the nitrogen content is high relative to the phosphate and potash contents the metabolism and physiology of the cacao trees suffer. On the whole the trees themselves must be relatively deficient in these elements but the limiting factor controlling tree metabolism is the deficiency of the element which is most lacking. Potash here fills this rôle. Any nutrient which tends to exaggerate the potash deficiency depresses the crop still further. The object of manuring cacao is thus to correct just which factor is limiting the speed of the tree mechanism which produces fruit. Without doubt on River Estate potash could be applied up to the point when phosphate would be the limiting factor and then both may be applied theoretically until it may even be beneficial to add nitrogen as well.

The Analysis of Wet Cacao Weight.

Improved nutrition may react on a plant, not only to increase the number of fruits produced but also to improve their quality. In cacao the size of the pod and also that of the bean depend very largely on nutrition for, bean number being the same, the larger pods come from the larger branches and for pods on the same cushion the one with the fewer beans usually produces the larger individuals. It is thus to be expected that any treatment which affects the factors limiting fruitfulness will be beneficial not only on pod number but on pod value. In certain localities McDonald has proved this to be the case following the application of phosphates.

The Analysis of Variance of wet cacao weights in this experiment is given in Table 10, where it will be seen that potash is beneficial. This Table must be compared directly with Table 6 since there are no natural yield records of cacao weights.

* Yates's correction.

Table 10.

Analysis of Variance of Wet Cacao Weight.*

Due to	D.F.	Sum of Squares.	Variance.
Main plot treatments	3	297.12	99.04
Nitrogen	1	28.12	28.12
Potash	1	264.50	264.50
N × K	1	4.50	4.50
Main plot Error	6	197.26	32.88
Total treatments	7	460.37	....
Phosphate	1	3.13	3.13
Inter. MP × SP Treatment	3	160.12	....
N × P	1	120.12	120.12
K × P	1	31.99	31.99
N × K × P	1	8.01	8.01
Sub-plot Error	12	690.66	57.56

The significance due to potash is such that the result would occur by chance less than once in 100 trials. Evidently potash has been more beneficial in increasing bean size than pod number. Phosphate has no effect at all.

It is evident from McDonald's results (*3rd Ann. Rept.* Chemical Section) that where potash is not limiting and phosphate improves the weight of cacao produced, the result may be due largely to the additional potash which is taken up by the cacao tree following applications of phosphate.

Addition of potash in this experiment has resulted in a total increase of 40 kgs. of wet cacao from 64 trees spaced 300 to the acre. This is equivalent to an increase in yield of 165 lb. of cured beans per acre, and in the first year of application.

The Effect on Bean Number.

Pollination experiments have demonstrated that at the time of fertilisation there may be considerable losses of potential seeds and that although the main causes may be genetic there is undoubtedly a nutritional effect. Unfortunately the large tree to tree variances mask any effect due to improved nutrition and probably it will be necessary to obtain previous records before reliable results can be obtained. The effect of treatments in this experiment is given in Table 11. Nitrogen and phosphate together depress mean bean number. This is probably another effect of unbalanced nutrition in the presence of a potash deficiency.

* Only treatments for simplicity.

Table 11.

Analysis of Variance of Mean Bean Number.

Due to	D.F.	Sum of Squares.	Variance.
Nitrogen	1	2.53	2.53
Potash	1	9.03	9.03
N × K	1	1.54	1.54
Error A	6	38.92	6.49
Phosphate	1	9.03	9.03
N × P	1	30.04	30.04
N × K	1	16.54	16.54
N × K × P	1	0.26	0.26
Error B.	12	98.63	8.22

The Effect on Percentage of Black Pods.

Blackening of pods, either at or just prior to maturity is usually associated with the presence of the fungal organism *Phytophthora faberi* causing one of the most serious diseases to which cacao is subject. Genetic resistance to this disease may or may not occur so far as present evidence goes but in any case there is a possibility as with most diseases, that resistance may vary with the balance or lack of balance in the nutrition of the host. The percentage of black pods has therefore been calculated for each sub-plot of the experiment and the analysis of variance for treatments is presented in Table 12.

Table 12.

Analysis of Variance of per cent. Black Pods.

Due to	D.F.	Sum of Squares.		Variance.
Nitrogen	1	47.50		47.50
Potash	1	124.00		124.00
N × K	1	26.35		26.35
Error A.	6	322.17		53.70
Phosphate	1	63.28		63.28
K × P	1	371.32		371.32
N × P	1	0.82		
N × K × P	1	11.20	971.90	69.42
Error B	12	959.88		

The actual data are summarised below by showing the infection on each treatment as a percentage of the infection on the unmanured plots.

	NK	K	N	C
+ P	81	87	66	78
− P	44	43	77	100

Though all treatments show some decrease in infection only the reaction potash × phosphate shows significance. This allows the following four treatments to be examined.

	+ K	− K
+ P	+22	− 1
− P	−55	+31

As there are eight half plots of each, the significance difference between these totals $=2.179\sqrt{69.42\times8\times2}=73$ approx.

Thus there appears to be greater infection when phosphate is applied with potash than when potash is applied alone and potash alone has a very significant depressing effect on the percentage of Black Pods.

Phosphate alone does not affect the result but in combination with potash annuls the beneficial effect of the latter. This presumably accounts for the lack of significance shown by potash treatments in the main plot analysis. These results must be accepted with reserve until confirmed by further observations, but they are suggestive. It is not unreasonable to expect a mineral deficiency, as marked as that of these plots in potash, to affect the susceptibility of the trees to diseases, but this is the first experimental evidence of any such effect in the case of Black Pod, and if it can be confirmed it must have important applications.

The Effect on Setting.

These observations are to some extent open to criticism. In the first place only a small actual percentage of blossoms set for any tree were recorded, since it is impossible from the ground to see any recent sets on the topmost branches. It follows that the data are comparative only and less variable than the true figures since there will tend to be more sets unregistered in the case of heavily setting trees than for poor setters. Secondly observations were only made at intervals of three weeks during the first year and many sets fail before they reach this age and are unrecorded.

As such the data for setting are presented as follows :

	NK	K	N	C	
+ P	798	867	790	597	3052
− P	539	753	479	568	2339
	1337	1620	1269	1165	

In each case there is greater setting in the presence of phosphate than without. It has more beneficial effects in the presence of nitrogen than potash since potash alone causes greatly increased setting and nitrogen tends to depress the effect of potash.

The Analysis of Variance is presented in Table 13.

The outstanding feature of the table is the significance of phosphate. To be significant Z in this case must exceed 0.7568. Potash is not significant in the analysis partly owing to the depressing effect of nitrogen. In actual practice it probably is more beneficial in this respect than appears for reasons advanced above. The results are interesting when examined in the light of final yield figures where there is no apparent effect of phosphate and where potash alone is beneficial. Probably both are more beneficial to setting than this analysis suggests.

The Effect on Wilting.

The actual figures for wilting are much greater than those for setting owing to the fact that wilting cherelles at the top of a tree can be seen and removed and in this respect the figures are probably more accurate than those for setting. The Analysis of Variance given in Table 14 suggests that both potash and phosphate are associated with increased wilting. Nitrogen to some extent allays the effects of potash but not of phosphate.

Table 13.

Analysis of Variance of Setting.

Due to	D.F.	Sum of Squares.		Variance.	Z
Main plot treatments	3	212.85		79.95	
Nitrogen	1	13.78		13.78	
Potash	1	124.03		124.03	0.6477
N × K	1	75.04		75.04	
Main plot Error	6	223.92		37.32	
Phosphate	1	247.53		247.53	0.9668
Inter. MP × SP Treatments	3	99.09	536.97	35.80	
Sub-plot Error	12	437.88			

Generally wilting is proportional to setting over most of the plots although the only treatment which shows significance is the interaction of nitrogen and potash.

	NK	K	N	C	
+ P	2550	3980	3662	2228	12420
− P	2682	2958	2833	1885	10358
	5232	6038	6495	4113	

Table 14.

Analysis of Variance of Wilting Cherelles.

Due to	D.F.	Sum of Squares.	Variance.
Main plot treatments	3	9485.5	3161.8
Nitrogen	1	220.5	220.5
Potash	1	1200.5	1200.5
N × K	1	8064.5	8064.5
Main plot Error	6	9383	1564
Phosphate	1	2145.1	2145.1
Inter. MP × SP Treatments	3	1530.4	650.46
Sub-plot Error	12	8226.5	

It is however important to note that the number of pods brought to maturity over a period of a year or more must depend on the distribution of setting in time as well as upon its total amount. Trees setting heavily at one time will also lose heavily at one time if adverse conditions supervene at the critical stages of development, whereas trees setting lightly over a longer interval will have fewer pods at the critical stage at any one time and may ultimately mature the larger crop without any greater inherent capacity to yield.

One possible benefit of suitable manuring is therefore a lengthening of the intervals over which setting may occur, and it is from this point of view that the foregoing observations have been made.

Summary.

1. The 4×4 Latin Square layout is capable of demonstrating significant increases of yield of the order of 30 per cent. during the first year of application of manures. This applies to established cacao of the usual genetically heterogeneous kind, provided that the trees are fairly uniform with respect to age.

2. Soil heterogeneity may account for more than half the total sum of squares. 'Error' may vary from $\frac{1}{6}$ of the total variance up to as much as $\frac{1}{2}$. In all cases it is large.

3. Precision may be increased fourfold by the use of previous records of a year in which the yields are highly and positively correlated with the yields of the experimental year.

4. Potash improves the bearing especially in the presence of phosphates.

5. There is some evidence that applications of potash improve bean weight as well as bearing capacity.

6. No effect of manures on bean number can be traced.

There seems to be a reduction of incidence of disease when potash is applied without phosphate. Since there are no previous records of this character and susceptibility may be a genetic factor, the findings must be accepted with caution.

8. Phosphate is generally significantly beneficial to setting. The effect of potash is somewhat obscured by the depressing effect of nitrogen applied in addition. Nitrogen alone improves setting, as was previously found for self-compatible trees, but here not significantly so.

9. No definite conclusions can be drawn with respect to the effect of manures on wilting of immature fruits.

STUDIES OF FRUITFULNESS IN CACAO.

VII.—Ovule Loss at Fertilisation.

By F. J. Pound.

Variation in number of beans between pods on the same tree was discussed in the First Annual Report (1931) (The Genetic Constitution of the Cacao Crop. p. 20 *et seq.*) and found to be large, so that while 10 to 20 pods taken at random served to define within prescribed limits the dimensions of the fruit, at least 30 were usually needed, and often more, to define bean number with similar precision. At the same time, dissections of ovaries at the open flower stage showed relative constancy in ovule number. Subsequent observations have established that there is a general tendency for the latter quantity to decrease in flowers on the finer branches. Other factors also induce variation, for even on the trunk, flowers may occur with a comparatively low number. Nevertheless, the total variance of ovule number is always less than that of bean number in the mature pod, and the difference indicates that an inconstant proportion of the ovules fail to develop into beans.

It was pointed out (*loc. cit.*) that among possible causes of loss, incomplete pollination, the operation of lethal genetic factors and physiological conditions have all to be considered. Any information allowing even a partial separation of the effects of these different sets of factors must bear directly on the analysis of final yield. *A priori*, either incomplete pollination or physiological fluctuations may be expected to be of irregular incidence, whilst the effects of lethal genes should be constant so long as the pollen parent is the same, but may vary with different crosses.

In the course of pollination studies it has been discovered that at least part of the failure of ovules can be detected within a week of pollination. After six days the majority of the ovules are turgid and have grown considerably, but a minority are shrivelled and even smaller than those in the open flower. In 50 per cent alcohol containing colouring matter (rum spirit) the latter turn brown within 24 hours, whilst the former remain plump and colourless (Fig. 7). There can be no doubt that these shrivelled ovules represent failures at the time of fertilisation. Consequently the loss at that stage can be determined with accuracy, and has been examined.

Methods.

Ovaries were obtained from controlled pollinations between a number of trees, some old and some young, some self-compatible and others not so. Flowers were pollinated by hand on the morning of opening and tagged with wool. They were not as a rule protected from self or other unwanted pollen, because it was calculated from the percentage of natural sets at the time, even on self-compatible trees, that there was little chance of contamination sufficient to affect the results. Of 50 flowers pollinated, in most cases at least 30 set if the cross was compatible at all, and the young fruits were picked at six days old and fixed in rum spirit for dissection. Dissection was carried out under a binocular microscope at a magnification of 25x, and the loculus was taken as the unit of observation. The numbers of sound ovules (above the line) and of failures (below the line) are set out loculus by loculus for one set of 30 ovaries in Table 15, in order to illustrate the kind of data obtained. The full records available for analysis comprise 47 such sheets.

Table 15.

Tree 24. Pollen from 18.

Loculi.					Ovary.	Total ovules.
10	10	8	8	8	44	
0	0	2	2	1	5	49
9	9	8	9	9	44	
1	1	1	1	0	4	48
7	8	8	9	10	42	
0	0	0	0	0	0	42
8	7	7	7	6	35	
0	0	1	1	2	4	39
9	7	7	8	7	38	
0	1	1	0	1	3	41
8	8	8	9	6	39	
0	2	0	1	2	5	44
9	9	9	8	7	42	
0	0	0	0	1	1	43
8	7	7	8	8	38	
0	1	1	0	0	2	40
7	8	8	8	7	38	
1	0	0	0	1	2	40
8	10	8	8	9	43	
0	0	2	0	0	2	45
7	6	7	5	7	32	
2	3	1	3	2	11	43
6	6	5	8	7	32	
2	2	1	0	1	6	38
4	8	5	8	6	31	
4	0	3	1	2	10	41
8	9	5	8	6	36	
0	0	3	1	3	7	43

Table 15.—*Contd.*

Tree 24. Pollen from 18.

Loculi.					Ovary.	Total ovules.
6/2	7/2	5/3	7/1	7/1	32/9	41
7/1	9/0	8/0	6/2	6/1	36/4	40
6/2	8/0	6/2	7/2	7/1	34/7	41
7/0	7/1	5/4	6/2	5/3	30/10	40
6/3	9/1	9/0	8/1	6/1	38/6	44
8/1	8/1	10/0	8/1	9/0	43/3	46
8/0	7/2	10/0	8/0	6/3	39/5	44
8/0	9/0	8/0	9/0	7/1	41/1	42
8/1	7/1	10/0	7/1	8/0	40/3	43
8/0	9/0	8/0	7/1	7/1	39/2	41
8/1	8/0	9/0	7/1	8/1	40/3	43
8/1	8/0	7/1	8/0	9/0	40/2	42
8/0	7/1	8/0	9/0	8/0	40/1	41
8/0	5/2	7/1	6/2	5/2	31/7	38
9/0	8/0	8/0	8/0	8/0	41/0	41
8/2	9/0	7/1	9/1	10/0	43/4	47

Total ovaries	....	30
Total Ovules	....	1,270
Total fail	....	129
Mean per loc.	....	8.47
Per cent. fail	....	10.16 per cent.

Fail per loculus.

0	1	2	3	4	5	6
68	48	23	9	2	0	0

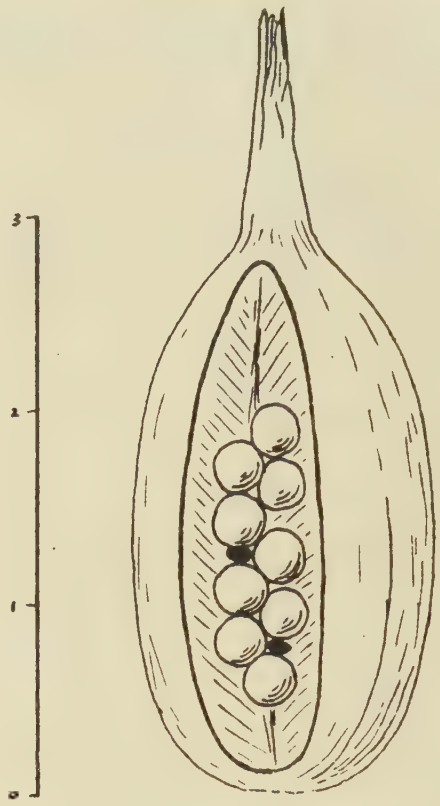

FIG. 7.—*Six day pollinated ovary with portion of wall removed to show one loculus containing nine sound and two failing ovules. Scale in mms.*

Gross Losses.

The percentage losses in all the crosses made are summarised in Table 16. Spaces marked 'x' represent incompatible matings, and incompatibility thus limits detailed investigation to the three self-compatible trees used. The most interesting point brought out is that remarkably different results are obtained from reciprocal crosses. Tree 8 is noticeable for giving consistently lower losses as a female parent than when used as male, but there is no similar consistency in the other cases. As they stand, the figures are unenlightening, save as to the complexity of the problem presented.

Variation in Ovule Number.

In making pollinations, it was not always possible to use constant proportions of trunk flowers and branch flowers, and consequently it is necessary before analysis of losses to determine how far samples of 30 ovaries are strictly comparable. 11 samples were dissected from Tree 24, and the distribution of ovules per loculus is shown in Table 17. From the analysis of variance, differences of 24 or more between sample totals are significant (P=0.05) and this value is exceeded in several cases. Groups of

samples within which no significant difference occurs are bracketed. The differences actually have little influence on the results, but allowance is to be made for them in certain cases when considering the distribution of failures per loculus.

Distribution of Losses.

Table 15, although chosen at random from the 47 sets of data available, exhibits several features common to most of the rest. In the first place it shows that the loculus is to some extent an independent unit, for in the five loculi of one and the same ovary all failures from zero to the maximum in the sample may occur, as exemplified by the 13th and 18th ovaries in the list. In other cases such failure distributions as 0, 0, 4, 0, 0, and 3, 2, 3, 0, 4 have been noted, and their frequence would seem to indicate that the loculus rather than the ovary is the unit of pollination.

On the other hand, there is a fairly close association between loss per loculus and loss per ovary. The four ovaries (Nos. 11, 13, 15 and 18) with the greatest total losses account between them for all the cases of four failures per loculus

Table 16.

Female parents.	Male Parents.											
	4	5	8	24	26	36	TC2	16	17	18	20	Mean per cent. loss.
4	x	x	9.59	19.52	13.49	x	x	x	x	x	x	14.20
5	x	x	12.81	6.67	12.07	x	x	x	x	x	x	10.52
8	6.67	7.93	5.88	3.10	3.89	....	....	2.61	9.08	....	2.54	5.21
24	11.73	14.68	13.32	12.41	13.81	10.62	13.81	9.83	10.67	8.05	9.12	11.64
26	9.88	11.06	10.80	9.07	8.10	10.47	12.46	....	....	....	4.63	9.56
36	x	x	12.89	12.44	11.69	x	x	x	x	x	x	12.34
TC2	x	x	....	17.82	....	x	x	x	x	x	x	17.82
16	x	x	36.45	25.98	29.97	x	x	x	x	x	x	30.80
17	x	x	9.60	14.78	12.02	x	x	x	x	x	x	12.13
18	x	x	....	40.41	....	x	x	x	x	x	x	40.41
20	x	x	28.46	24.72	17.30	x	x	x	x	x	x	23.49
Mean	9.43	11.22	15.53	16.99	13.59	10.55	13.13	6.22	99.88	8.05	5.43	

Table 17.

Ovs. per loc.	Male Tree Number.										
	26	36	20	8	4	TC2	5	16	24	17	18
6	2	0	0	1	1	1	2	0	0	1	1
7	19	12	11	11	16	12	13	8	8	9	7
8	104	96	98	92	73	83	76	85	82	71	82
9	23	38	35	36	54	44	49	52	50	61	41
10	2	4	6	10	6	10	10	5	10	8	19
Total	1,204	1,234	1,236	1,243	1,248	1,250	1,252	1,254	1,262	1,266	1,270

Analysis of Variance.

Due to	D.F.	Sum of Squares.	Variance.
Between Samples	10	27	2.7
Within Samples	1,639	816	0.498
Total	1,649	843	

and the majority of the cases of 3. With this is coupled the fact that the distribution of losses *per ovary* is discontinuous between 7 and 9, so that two distinct failure series may arbitrarily but fairly be recognised, as shown in Table 18.

If discontinuity of distribution were never more marked than in this example it might scarcely merit attention, but in some cases it is much more conspicuous, whereas in others it does not appear at all. Selected examples of both kinds are given in Table 19.

It is a fair conclusion that in certain cases we have to distinguish between what may be called "normal" and "abnormal" losses. The latter may be due to inadequate pollination, or to physiological factors preventing an adequate number of pollen tubes from reaching the micropyles. They can scarcely be caused by the direct action of genetic factors, and in discussing the possible action of the latter they will be discarded. Meanwhile their occurrence goes far to explain the large variance of bean number in ripe pods, and when "abnormal" ovaries are discarded the variance of sound ovules in those remaining becomes comparable with that of pod dimensions.

Types of Failure Series.

The distribution of failures per loculus in all samples from Tree 24 is given in Table 20, where, as in Table 17, groups of samples within which no significant difference occurs are bracketed. In this case differences of 38 or more between sample totals are significant (P=0.05). The sample set with pollen of Tree 17, however, contained (as shown in Table 17) rather more ovules than that set by pollen of 16 and significantly more than that set by pollen of 20, and since more ovules will mean more losses if the percentage is the same, this sample may be put with those to the left of it. This is interesting, because the four trees, 16, 17, 18, and 20, are sibs of Criollo ancestry, and all the rest (except one) sibs of 24.

Pollen of Tree 4 and that of Tree 8 appear to be giving results significantly different from the rest of their class, but the differences disappear when allowance is made for "abnormal" losses. There are thus in reality only two classes, and they show two distinct types of failure series. In the first the greatest frequency of failures occurs at zero per loculus, and in the second it occurs at one per loculus. The masking effect of variability in ovary samples is seen again in the case of the sample pollinated by Tree 26, which had significantly fewer ovules than any other sample and probably for this reason shows as many failures at zero as at one per loculus. Apart from the two cases, however, the effect is not serious, and it will be noted that there is no correlation between the bracketed groups of Table 17 and those of Table 20.

When allowance is made for "abnormal" losses, the percentage losses in the several samples appear as set out in Table 21.

Table 18.

	Failures per loculus.					
	0	1	2	3	4	
Frequency, 26 ovaries	66	43	17	4	0	Mean loss 8.05 per cent.
Frequency, 4 ovaries	2	5	6	5	2	Mean loss 24.24 per cent.
Frequency total	68	48	23	9	2	Mean loss 10.16 per cent.

Table 19.

Cross.	Failures per ovary.													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
24 x 18	2	3	5	4	4	3	2	3	....	1	2	1	....	
24 x 17	....	....	2	7	6	2	5	4	....	....	2	....	....	2
8 x 16	6	12	8	2	....	....	....	1	....	....	1			
24 x 4	....	1	2	6	5	4	6	3	1	2	....			

Table 20.

Failures per loculus.

Ovules Fail.	Male Tree Number.										
	16	18	20	17	4	TC2	36	24	26	5	8
0	67	68	67	54	54	43	43	37	42	31	28
1	49	48	43	50	59	56	50	58	42	58	47
2	28	23	27	31	26	36	28	30	41	35	35
3	6	9	8	10	9	8	20	16	17	17	26
4	0	2	5	4	2	6	8	6	7	6	10
5	0	0	0	1	0	1	1	3	1	3	4
Total	123	129	141	163	146 ?	181	203	205	208	218	255

Analysis of Variance.

Due to	D.F.	Sum of Squares.	Variance.
Between Samples	10	116	11.6
Within Samples	1,639	1,985	1.211
Total	1,649	2,101	

Table 21.

	Young Criollo Trees.				Self-incompatible Trees.				Self-compatible Trees.		
	18	20	16	17	4	5	36	TC2	24	8	26
Per cent. loss in "Normal" ovaries	8.05	9.12	9.83	10.67	11.73	14.68	10.62	13.81	12.41	13.32	13.81
Per cent loss in "Abnormal" ovaries	24.24	23.90	None	27.56	None	35.12	30.73	35.71	30.26	31.90	30.86
	No significant difference.				No significant difference.						

From this re-arrangement it also appears that there is no linkage between pollen compatibility factors and any lethal or physiological factors that may be operative.

Comparison with Poisson Series.

Failures due to a single factor, or a group of factors, acting with a constant frequency should theoretically be distributed in a Poisson Series, and the observed losses of ovules at fertilisation may be examined in this way for agreement with the hypothesis that the failures are largely due to genetic causes. In order to avoid the complications of varying ovule number per loculus, the "normal" failures may be tabulated for just those loculi which contained eight ovules. This has been done for the first type of failure in Table 22. The mean failure per loculus is 0.813 ovules, and the "Expected" frequency is the Poisson Series calculated for M=0.8.

Table 22.

Failures per loc.	Pollen Parent.				Total.	Expected.
	16	17	18	20		
0	32	26	37	42	137	134.8
1	31	24	23	22	100	107.8
2	19	8	8	14	49	43.2
3	3	4	1	2	10	11.6
4	0	1	1	2	4	2.4
Total					300	299.8

In testing for Goodness of Fit (6) $X^2=2.6$, $n=3$, whence P lies between 0.4 and 0.5, showing that the observed frequency is not significantly different from the Poisson series.

Data for the second type of failure are similarly arranged in Table 23. In this case the mean failure is 1.04 per loculus.

$X^2=2.0$, P lies between 0.7 and 0.8, and there is no reason to suspect the hypothesis that the figures fall in a Poisson series with $M=1.04$.

Other Failure Series.

Of the figures obtained from pollinations on Tree 8 and Tree 26, only those have been analysed which pertain to samples not significantly different from each other in ovule number. These are presented in Table 24, and brackets again indicate groups within which no significant differences occur, the significant difference between totals being in this case 34 or more.

Table 23.

Failures per loc.	Pollen Parent.							Total.	Expected.
	4	5	36	TC2	24	8	26		
0	26	19	31	25	21	13	30	165	175
1	29	28	28	29	30	25	23	192	182
2	12	14	8	20	8	13	19	94	95
3	6	7	3	3	6	2	9	36	33
4	0	0	0	3	0	1	1	5	7
5	0	1	0	0	0	0	0	1	1
Total								493	493

Table 24.

Male.	16	24	20	20	26	8	8	24	36	4	5	TC2
Female : Loss per loc.	8	8	8	26	26	8	26	26	26	26	26	26
0	110	106	109	91	75	81	60	61	51	59	44	35
1	31	33	26	50	49	38	44	49	56	46	52	61
2	7	8	6	7	17	20	21	24	27	26	30	36
3	2	3	5	1	8	7	13	12	13	14	15	9
4	0	0	2	1	1	2	1	4	3	4	7	7
5	0	0	2	0	0	1	1	0	0	1	2	2
Total	51	58	71	71	111	112	134	149	161	161	195	198

Analysis of Variance.

Due to	D.F.	Sum of Squares.	Variance.
Between Samples	11	198	18.00
Within Samples	1,788	1,705	0.954
Total	1,799	1,903	

(6) Fisher, R. A.—" Statistical Methods for Research Workers."

Correcting for "abnormal" losses the data may be re-arranged as in Table 25.

Table 25.

Cross.	Per cent. loss ("Normal" ovaries).	Per cent. loss ("Abnormal" ovaries).
16 on 8	2.61	18.89
24 on 8	3.10	11.89
20 on 8	2.54	30.30
20 on 26	4.63	19.14
8 on 8	5.88	22.87
26 on 26	8.10	None
8 on 26	10.80	None
24 on 26	9.07	28.24
36 on 26	10.49	28.57
4 on 26	9.88	30.43
5 on 26	11.06	27.01
TC2 on 26	12.46	27.07

There are here *three* types of failure series, of which the two with higher losses correspond to those found in samples from Tree 24. The third, showing exceptionally low percentages of loss in "normal" ovaries is new. Combining Table 21 with Table 25 the average "normal" failures in the three series are 3.75 per cent., 9.56 per cent., and 12.66 per cent. respectively.

Discussion.

The results of this partial analysis of ovule failure at the time of fertilisation tempt speculation, but scarcely warrant it, so far as any detailed hypothesis is concerned. The close approximation of failure series to certain distinct patterns, and the close agreement between those patterns and Poisson series with the same mean, leave little room for doubt that genetic lethal factors are operating in a great many of the cases investigated. A few failures would be expected from mechanical and physiological causes even in the total absence of lethal genes, and it is possible that the first series (average loss 3.75 per cent.) represents those. In the second series the average loss is 9.56 per cent., or 5.81 per cent. higher than the first; the approximation to 6.25 per cent. (1/16) is suggestive but may well be accidental. The third series is 3.10 per cent. (almost 1/32) higher still. Beyond that there is not yet enough evidence to proceed.

Embryo abortion at a later stage has not been discussed, but it is worth noting that the losses at fertilisation found in this work are generally lower than the total losses calculated (on different trees) by subtracting average bean number from average ovule number (*First Ann. Rept.* 1931. p. 22). It is probable therefore that appreciable further losses occur at later stages.

So far as variance of bean number is concerned, which is the important economic consideration, variance of ovule number, normal loss at fertilisation and embryo abortion will all undoubtedly contribute to it, but the greatest factor seems to be the abnormally high loss that occurs at fertilisation in certain ovaries, irregularly distributed. For this the most likely explanation is incomplete fertilisation due either to an insufficiency of pollen grains, or to physiological factors of irregular incidence that hinder the growth of pollen tubes.

Summary.

Variation in bean number per pod had previously been found to be unexpectedly large and was traced to inconstant losses either at the time of fertilisation or later. By dissections of six day pollinated ovaries the losses at fertilisation have been examined for some trees.

Losses appear to be of two kinds, one relatively constant and possibly due to the operation of lethal genetic factors and the other irregular and probably physical or physiological in origin.

Further losses are known to occur from embryo abortion but these were not examined.

ON THE GERMINATION OF CACAO BEANS WITH SPECIAL REFERENCE TO STORAGE AND TRANSPORT PROBLEMS.

By E. E. Pyke.

Introduction.

The cocoa bean differs from the seeds of most plants in having practically no resting period between ripening and germination. The beans usually exhibit spontaneous germination in the pod within five to eight weeks after ripening, unless they have been previously liberated by decay of the pod wall, or unless spoiled by fungal attack or desiccation, both of which cause rapid loss of viability. Also, beans apparently healthy, unaffected by desiccation or fungal attack, seem to have the power of effective germination impaired by continued storage at tropical temperatures, exceeding about eight weeks. In order to make possible the collection of new varieties of cocoa in the less accessible parts of South and Central America, with subsequent germination of the beans, methods would need to be available for preserving the pods for some weeks at tropical temperatures, even if low temperature transport were possible in shipping to England.

Experiments to improve the keeping powers of cocoa pods were conducted along the following lines :—

(*a*) Reduction of the rate of desiccation.
(*b*) Reduction of the rate of fungal decay.
(*c*) To find the best temperature for storing the pods.
(*d*) To find the best way of securing good germination from stored beans.

For (*a*) the treatment of pods with such agents as paraffin wax, vaseline, rubber latex, and with various wrappings was tried. The heading (*b*) has really two aspects, firstly the reduction of the incidence of fungal infection, and secondly the reduction of the rate of decay when fungi are present. The effects of care in selecting the material, and of the use of antiseptic treatments, were observed in connection with the first aspect, and the second was studied by storing pods at different temperatures, this being part of the more general problem of the effect of temperature, such as chilling and rate of senescence, included under heading (*c*). Under heading (*d*), the effect of different media and temperatures upon the rate and final percentage of seed germination was investigated.

Methods.

Collection of Pods.—Pods for the storage trials were in most cases carefully hand-picked, with precautions against bruising, and were brought by car from the estate to the laboratory. Some samples were purposely exposed to a reasonable amount of bruising and abrading on the journey, while others were for comparison treated with the utmost care throughout. In later trials, when the importance of latent or dormant infections was realised, each pod was carefully scrutinised before and after picking especially in the region of the stem, for the presence of these blemishes.

Storage.—The pods were stored on the laboratory bench to represent average tropical temperatures, and in storage chambers of the Low Temperature Station, I.C.T.A., to provide temperatures of 45°F, 50°F, 53°F, 60°F, and 70°F. respectively. Throughout this paper the laboratory storage temperature is designated as 80°F, this being a fairly accurate diurnal mean.

Germination Tests.—All the germination trials were made in shallow boxes, and in most, the medium used was washed, fairly coarse calcareous sand. In the study of the higher ranges of temperature the boxes were put in various greenhouses, and for the lower temperatures, chambers of the Low Temperature Station were used. In assessing germination, a count was made after six or seven days, the beans being taken up, and the germinated beans divided into classes according to the lengths of the radicles formed. In this way a roughly quantitative estimation of the potential germination power was formed, since it was found by trial that in fresh healthy batches of beans the germination count after seven days in a fairly warm temperature, (a greenhouse of mean temperature of 84°F.) approximated to the final effective germination figures. It was also found that with stored beans which showed in the final tests a partial reduction of viability, due to causes other than obvious fungal attack, the germination up to seven days was frequently good, sometimes almost normal, and that failure occurred at a later stage and involved the death and decay of the radicle. This first count is referred to as the *potential germination*, and the count made on the same beans four to five weeks after sowing provides the *effective germination*. In order to check the validity of this estimate of the effective germination, random batches of seedlings from different trials were potted up and kept in the nursery for several months to observe their subsequent behaviour. It was found that all the plants developed normally, with no further mortality.

Experimental Results.

(1) Laboratory Storage of Untreated Pods.—Ripe pods were picked and stored on the laboratory bench. Samples were selected at random at successive intervals after picking, and the beans were planted.

Number of days stored—0, 2, 4, 8, 14.

Percentage effective germination—98, 97, 100, 94, 95.

(2) Effect of Low Temperature Storage on Germination. (*Preliminary experiment*).—Three sets of six pods each were used; one set was kept in the laboratory, another set in a storage chamber at 50°F, and the third set in a chamber at 45°F. Single pods were taken from each set for germination at intervals of 2, 3, 5, 6, 7 and 9 days. Results are presented in Table 26.

Table 26.

Temperature.	Percentage effective germination.					
	2	3	5	6	7	9 days.
80°F.	98	100	95	90	93	94
50°F.	0	0	0	0	0	0
45°F.	0	0	0	0	0	0

It is apparent that whereas nine days tropical storage is not long enough to produce diminished germination, even two days at 50°F. or 45°F. is sufficient to destroy completely the viability of the beans. The lethal effect of these temperatures was verified in a later experiment.

2nd Experiment.—The temperatures used were 80°F, 70°F, 60°F, and 53°F. Beans were put out for germination tests after 2, 5, 8, 12 and 20 days storage respectively. Two pods were used for each test, giving a germination sample of 60 to 80 beans. Results in Table 27.

The relative humidities were :—

at 53°F.—80 to 90 per cent.
60°F.—80 per cent.
70°F.—70 to 75 per cent.
80°F.—50 to 90 per cent.

Table 27.

Temperature.	Percentage effective germination.					
	2	5	8	9	12	20 days.
80°F.	96	85	99	....	84	12 ; 69 (mean 40)
70°F.	93	58	100	....	40	73
60°F.	66	86	92	....	74	91
53°F.	27	3	20	9	(discontinued after 9 days)	

The poor germination resulting from only a short storage period at 53°F. is noticeable.

Condition of the Pods.

5th day.—The pods at all temperatures were firm and appeared fresh.

9th day.—At 53°F., the pods were slightly flaccid and showed brown discolouration on the ridges.
At 60°F., the pods were firm and fresh.
At 70°F., the pods were slightly flaccid, two were developing fungal infections, at the stalk end and the tip respectively.
At 80°F., the pods were very wilted and flaccid.

20th day.—At 60°F., the pods were still firm and fresh.
At 70°F., the pods were brown and dry.
At 80°F., the pods were dry, shrunken and hard. The beans were desiccated, and in one pod they were densely covered with a dark fluffy mycelium.

The value of 60°F. storage is apparent from this experiment. At this temperature alone the pods were perfectly fresh, and gave beans of normal germination, after 20 days.

(3) The Effect of the Medium upon Germination.—Four different media were tried :

(1) A fairly coarse-grained calcareous beach sand, washed with fresh water.
(2) A high-grade non-calcareous silver sand, of "horticultural" quality, slightly finer than (1), imported from England.
(3) A mixture of dried and sieved cow-dung, 2 parts by volume, with the sand No. (1), one part by volume.
(4) Similar to medium (3), but with sifted vegetable compost replacing cow-dung.

The germination boxes were lightly watered and kept in a greenhouse of temperature range 76°F. to 86°F. Samples of about 150 beans were used for each test. The germination counts were made after six days and after 26 days, and the results, expressed as percentages are set out in Table 28.

Table 28.

Potential and Effective Germination of Beans in four different Media.

Medium.	Germination at 6 days.	Germination at 26 days.
	per cent.	per cent.
Calc. sand	48	92
Silver sand	25	97
Dung and sand	48	87
Compost and sand	33	79

The two grades of sand both gave better ultimate germination than the other media, although the beans in sand No. (2) showed some lagging at the beginning. As sand No. (1) was the cheapest and most readily available medium, it was standardised in all the subsequent trials.

(4) The Effect of Temperature upon Germination.—Media Nos. (1) and (2) of the above experiment were used in germinating beans in houses of different mean temperatures. The only constant temperature available was that of 70°F. in one of the Low Temperature chambers ; the higher temperatures were obtained in two different greenhouses of respectively moderate and high, relatively speaking, average temperatures. From thermometer readings the average

diurnal temperatures in the germination media were estimated at 79°F. and 86°F. respectively. Samples of 150 beans were used in each test. The results are given in Table 29. (It is to be noted that the 79°F. trial is the same one as in the previous experiment.)

Table 29.

Potential and Effective Germinations at Different Temperatures.

Temperature.	Germination at 6 days.		Germination at 26-31 days.
	per cent.		per cent.
70°F.			
Medium (1)	9.5	5.5	87
Medium (2)	1.3		86
79°F.			
Medium (1)	48	37	92
Medium (2)	25		97
86°F.			
Medium (1) only ...	75		96

A marked retardation of the germination rate is evident at 70°F.

2nd Experiment.—Fresh beans were germinated at the following temperatures :—

(1) 60°F. Constant (low temperature chamber).

(2) 70°F. do. do.

(3) 79°F. Mean (greenhouse ' A ').

(4) 85°F. Mean (greenhouse ' B ').

(5) 90°F. Constant (electric propagator).

Germination counts were made after seven days, 14 days, and in the 60°F. trial only, after 40 days. At each count the number of beans with radicles over one-half inch long was noted. The results are presented in Tables 30 and 31.

Table 30.

Germination at Temperatures from 60°F. to 90°F.

Temperature.	Germination after 7 days.		Germination after 14 days.	
	Total.	with roots over ½ inch long.	Total.	with roots over ½ inch long.
	per ct.	per cent.	per ct.	per cent.
60°F.	0	0	56	0
70°F.	0	0	96	85
79°F.	32	0	94	85
85°F.	89	7	97	97
90°F.	96	51	96	96

Table 31.

Germination at 60°F. after 40 days. Figures are numbers of beans.

Germination.	Healthy beans.	Decayed beans.	Percentage decayed.
			per cent.
(*a*) no apparent germination	0	18	100
(*b*) radicle just breaking through the testa	0	7	100
(*c*) radicle 1/8 to ¼ inch long	2	17	90
(*d*) radicle ¼ to ½ inch long	20	4	17
(*e*) radicle over ½ inch long	30	1	3
Totals	52	47	
Total germination		81 per cent.	
Total effective germination		52 do.	

The healthy germinated beans from the 60°F. trial were re-sown and put in a greenhouse at tropical temperature. Growth was rapid and the beans soon developed into seedlings of normal appearance. Sections of the beans with decayed radicles showed that the cotyledons were involved in more or less discoloration and decay, resembling that in beans stored in the pods at 50°F. and 45°F. From the table it is seen that decay occurred mostly in the less vigorous germination classes. Fungal attack was not observed in any of the beans in this experiment, either at the high or the low temperatures. The partial mortality at 60°F. may be possibly due to a chilling effect which upsets the food supply mechanism to the radicles. This does not occur in beans stored in the pods, at least not up to 50 days storage, according to a later experiment, but in another experiment in which beans, previously germinated to different extents at tropical temperature, were exposed to 60°F. a high mortality was recorded in the just germinated beans, and a lower mortality in those which had been germinated for 10 to 15 days. Older cocoa seedlings seem to be able to withstand 60°F. for periods of a week or so ; longer periods could not be fairly tested due to the absence of daylight in the chamber.

(5) The Effect on Germination of Soaking Cocoa Beans before Sowing.

(*a*) *Fresh Beans.*—The treatments consisted of soaking the fresh ripe beans for one hour in water at the temperatures 86°F., 95°F., and 104°F., (30°C., 35°C., and 40°C.) respectively, using unsoaked beans as controls. The beans were sown in lightly watered sand and kept in a moderately warm greenhouse. The results are given in Table 32.

Table 32.

Germination percentages of Fresh Beans, water-soaked for one hour. 150 Beans in each Test.

Treatment.	Percentage Germination after		
	6 days	23 days.	39 days.
	per cent.	per cent.	per cent.
Soaked at 86°F.	63	99	100
Do. 95°F.	65	97	98
Do. 104°F.	61	97	97
Controls, unsoaked	47	94	93

The soaking at all three temperatures showed a marked stimulating effect upon the initial germination rate, but had little effect on the final result.

(*b*) *Stored Beans.*—The experiment was repeated, using instead of fresh beans, beans from pods which had been stored in the laboratory for 22 days. An additional soaking treatment of 12 hours in water at laboratory temperature was introduced. The results are given in Table 33.

Table 33.

Germination Percentages of Beans Stored for 22 days Soaked and Unsoaked. 150 beans in each Test.

Treatment.	Percentage Germination after	
	6 days.	41 days.
	per cent.	per cent.
Soaked at 86°F. (1 hour)....	35	11
Do. 95°F. do.	35	11
Do. 104°F. do.	35	10
Controls, unsoaked	64	30
Soaked overnight at laboratory temp.	70	62

The results of the one hour's soaking treatment are quite different in the two experiments. With the fresh beans there was a marked stimulus to rapid germination, but the final germination figures were of the same order as those of the controls. With the stored beans however, both the initial and the final germination counts for the one hour's soaking were much lower than those for the controls. With an overnight soaking however, the beans were about equal to the controls in initial germination, and very much better in final germination. In comparing the two trials it must be noted that the second batch was exposed to a rather higher temperature during the early stages of germination, sufficient no doubt, to account for the more rapid germination of the controls of the 2nd trial compared with those of the first.

Germination tests were also made with beans from pods stored in the laboratory for 32 days. The pods from which beans were taken were divided into three classes according to condition :—(*a*) those badly attacked by fungal decay, (*b*) those lightly infected only, and (*c*) those apparently unaffected. In the first group the pods were extensively rotted, and in all of them fungus had penetrated to the interior and formed a dense weft upon the beans, which were also rather desiccated. In these circumstances it is surprising that fairly good germination was shown. For comparison, the germination figures of beans from lightly infected and uninfected pods are also given in Table 34.

Table 34.

Germinations of beans stored in laboratory for 32 days from heavily infected, lightly infected, and uninfected pods. 100 beans in each test.

	Germination after 6 days.	
(*a*) *Beans from heavily infected pods.*	Potential.	Actual.
	per cent.	per cent.
Soaked at 86°F. (1 hour)....	77	47
Soaked at 95°F. (1 hour)....	73	51
Control, unsoaked	76	42
Soaked overnight at laboratory temperature....	69	53
(*b*) *Beans from lightly infected pods* (not soaked)	55	53
(*c*) *Beans from sound pods* (not soaked)	100	100

The figures in the first column, of potential germination, were computed by adding the counts of *living* germinated beans after six days, and those which had started to germinate but had died before the sixth day inspection.

Unfortunately owing to lack of accommodation this trial had to be discontinued before final germination counts could be made. The results indicate however that beans from pods which have undergone extensive fungal attack in storage, show a diminished viability, manifested especially by a tendency to decay during the early stages of germination. The results from soaking before sowing seem inconclusive. With the beans stored for 22 days a short soaking was harmful, but soaking overnight seemed beneficial. On the 32 days sample, admittedly of low grade beans, neither short nor long soaking showed any appreciable effect.

Beans from only lightly infected pods did not give any better initial germination than those from badly rotted pods. Only the two pods which had withstood 32 days' storage without any trace of fungal attack gave beans of normal germination power. In this connection it is

significant that in all the infected pods, even when the external lesions were small, fungi had penetrated to the internal cavity and fermentation changes in the mucilage of the beans had been set going by 32 days, resulting in a musty-rancid smell, in which the aromas of acetic and butyric acids and various esters could be detected. It thus seems that the first step in maintaining the viability of beans stored in the pods, is to avoid fungal infection of the pod wall. This was verified in later trials.

(6) Experiments using Stored Pods with Antiseptic and Anti-desiccation treatments. It was observed in the early trials with pods stored in the laboratory, that both wilting and fungal decay of the pod walls proceeded rapidly together. Extensive fungal attack greatly hastened the rate of wilting. Simply dipping the pods in melted paraffin wax appeared to do little to check either process.

Two experiments in collaboration with Dr. Wardlaw and Mr. Leonard, of the Low Temperature Station, were made, using pods smeared with vaseline, or dipped in raw rubber latex, as anti-desiccation treatments, with and without antiseptic dips in 0.2 per cent. mercuric chloride solution. The pods were stored at 80°F., 70°F., 60°F., 50°F., and 45°F., and samples were taken out at ten day intervals, up to 50 days, for germination tests. As these experiments have been published elsewhere (7), it is only necessary to give here a brief account of the results :—

(*A*) *Fungal Wastage.*—(*a*) At 70°F. and 80°F. respectively fungal wastage became conspicuous between the tenth and twentieth day in storage and thereafter increased rapidly in intensity.

(*b*) Mechanical injury previous to storage greatly increased the liability to rotting.

(*c*) In some pods, particularly those subjected to mechanical injury, the hyphae of *Botryodiplodia theobromae* had penetrated through the pod and had formed a weft round the beans, destroying the mucilage by 20 days. Such effects were intensified after 30 days. The beans of these pods when sectioned, however, appeared normal and uninjured.

(*d*) With pods bruised or injured during harvesting and transport, or in which sub-epidermal, latent, or dormant infections had been established previously, no effective control of fungal wastage was obtained by any of the several disinfectant treatments applied.

(*B*) *Desiccation of the pods.* (*a*) *Untreated pods.* At 80°F., desiccation was rapid and progressive, amounting to 46 per cent. of the original weight after 20 days, and 81 per cent. after 50 days. At 70°F. the rate of desiccation was still considerable, being 23 per cent. and 70 per cent. after 20 and 50 days respectively. At 60°F. and 50°F. desiccation was much slower, averaging less than half the rate at 80°F.

(*b*) *Pods treated with Vaseline.* The rate of loss of weight in the pods smeared with vaseline was highly variable and depended upon whether fungal wastage developed or not. When pods were extensively attacked by active parasites such as *Botryodiplodia theobromae* and *Gleosporium luxificum*, the rate of loss of weight after 10 or 20 days storage was not less than that in untreated pods, but in pods which escaped attack, or were only slightly infected, the loss of weight was small at 80°F. (4.5 per cent. after 20 days and 17 per cent. after 50 days in one case) and was even less at 70°F.

(*C*) *Germination of the Beans.*—(*a*) 45°F.—No signs of germination were shown by any of the samples even after only ten days' storage. The cotyledons were discoloured almost to the centre.

(*b*) 50°F.—A low percentage of incipient germination was evident up to 30 days, but the beans perished when the radicles had made only a few millimetres growth. In sectioned beans the radicles appeared fresh and healthy, but there was progressive browning of the cotyledons from the outside as storage was prolonged. It thus seems that 50°F. does not directly kill the resting embryo, but in some other way, possibly by disorganising the supply of food material from the cotyledons, prevents effective germination.

(*c*) 60°F.—The pods at this temperature were not treated against desiccation, but nevertheless kept fairly fresh during the whole of the trial. Germination was good up to 40 days, and was fair at 50 days.

(*d*) 70°F.—*Untreated Pods.*—Effective germination was fairly good up to 30 days, but after that fell off rapidly.

Vaselined Pods.—Individual pods which escaped fungal infection gave beans of good germinating power after 50 days.

(*e*) 80°F.—*Untreated Pods.*—Germination was fair up to 20 days, but beyond that time was negligible.

Vaselined Pods.—Sound pods gave good germination up to 30 days, and a few fairly good germination at 40 days. At 50 days, however, beans which were perfectly fresh in appearance failed to germinate well, so that a deterioration was evident apart from that caused by desiccation and fungal attack.

It is clear from these experiments, that the keeping powers of the pods, and the resultant viability of the beans, depends primarily upon the absence of latent or dormant infections in the pod walls, or the stalks. Their development is not effectively prevented by any antiseptic treatment, and if initially present the pods are rapidly destroyed unless the temperature is kept down to about 60°F. In the two experiments summarised above, no special precautions were taken against the inclusion of pods with latent infections, so that the storage behaviour of such pods could be studied. By carefully selecting only sound pods, however, smearing them with vaseline to reduce desiccation, and wrapping in greaseproof paper or cellophane to minimise

(7) Pyke, E. E., Leonard, E. R. and Wardlaw, C. W.—"On the Viability of Cacao Seeds After Storage." *Tropical Agriculture*, XI, 12, 1934.

contamination by contact with diseased pods, pods may be preserved for 40 to 50 days at temperatures of between 70°F and 80°F. A batch of sound pods so treated was collected by the writer and taken to England from Trinidad in the ordinary baggage room of a ship, with no artificial cooling. In spite of hot weather on the voyage, and for part of the time in England fungal wastage and consequent loss of weight, was not excessive, and cases of good germination were obtained at the Royal Botanic Gardens, Kew, after 65 days from the time of picking.

Thus an aseptic rather than an antiseptic technique should be aimed at in the collection and handling of pods for storage. There may be circumstances in which this counsel of perfection cannot be followed, such as when it is required to collect from one particular tree which may not bear any entirely unblemished pods. In these cases prolonged storage could only be successful by reducing the temperature to a level at which the parasites would remain dormant or would develop relatively slowly. In this connection the temperatures 53°F. to 54°F. deserve special attention, as this temperature range is used in banana transport from ports of South and Central America to Europe. Thus if cocoa pods would stand this temperature for a period up to 20 days without the beans being killed, even those showing incipient decay could be successfully transported. Previous experiments have indicated that viability cannot be maintained at 50F°. but is normal at 60°F. The critical temperature must therefore lie between 50°F. and 60°F. The experiments to be described suggest that 53°F. represents about this threshold temperature. In this connection it must be noted that 53°F. is the minimum rather than the mean in banana storage, and fluctuations upwards of 1°F. to 1½°F. are possible according to the position in the hold and the setting of the temperature regulating mechanism. The results of storing cocoa pods at this nominal temperature are hence rather variable; it is assumed that when a considerable increase of effective germination occurred the storage temperature was a degree or so above 53°F. All the experiments made so far at 53°F. have been on freshly picked pods or on pods stored not longer than 12 days at tropical temperature. Further trials are proposed on pods stored for longer periods at high temperature, in which fungal rotting is actively proceeding.

(7) Experimental Results at 53°F.

(1) Freshly reaped pods, *i.e.*, reaped and put in storage the same day, were held at 53°F. in a chamber of the Low Temperature Station.

Results.—Two pods, *i.e.*, 60 to 80 beans in each test.

Time of storage	2	5	8	9	days.
Percentage effective germination	27	3	20	9	%

This may be compared with the corresponding figures for 60°F. and 70°F. in the same experiment.

60°F. effective germination	66	86	92	74%
70°F. do.	93	58	100	40%

(2) Pods were kept at laboratory temperature (about 80°F.), for five days before transfer to 53°F.

Results.—Time of storage at 53°F. ....10 15 days.
Percentage effective germination 8 9 %

These figures are based on the healthy seedlings alive to five weeks after sowing. The initial germination rate was much higher. Germination in this experiment was conducted respectively with, and without, a preliminary soaking in warm water (35°C. for one hour) before sowing. The warm water treatment seemed to give an initial fillip to germination, but the advantage was not maintained (Table 35).

Table 35.

Percentage germination after	Time of storage at 53°F.			
	10 days		15 days	
	soaked	un-soaked	soaked	un-soaked
4 days	40	14	....	....
7 days	60	53	31	21
17 days	10.5	15	....	....
4 to 5 weeks	7	9.5	10	8.5

The effective germinations in this experiment were consistent, and, averaging all the batches, were about nine per cent. after 10 to 15 days at 53°F.

(3) The results of the last experiment were confirmed by another trial in which previous laboratory storage of three days, nine days, and 14 days respectively, were tested in conjunction with 17 days storage at 53°F., and also with 17 days storage at 50°F.

The results (from 50 beans in each test) are given in Table 36.

Table 36.

Time at 80°F.	Storage at 53°F. eff. germination.	Storage at 50°F. eff. germination.
14 days	0	0
9 days	0	0
3 days	22	12
0 days	6	0

Thus three days "quailing" gave appreciable resistance to storage at 50°F. even, and a much higher germination figure was recorded for 53°F. than in previous trials.

(4) Some experiments were made on beans removed from the pods, and exposed to low temperatures, including 53°F. In some trials beans were allowed to germinate to different extents at laboratory temperature before

exposing to chill, in others partial desiccation was permitted. Results were uniformly poor; the lower temperatures seemed to have a greater lethal action on the exposed beans, than upon beans kept in the pods. The method does not promise practical results and is inconvenient.

In the latest experiment the effects of short periods of tropical pre-storage were investigated with more precision. This was insured by (*a*) using pods from one tree only, and (*b*) by taking a random sample of six pods for each germination test yielding 150 to 200 beans which were divided into four equal lots and germinated in four boxes. Five periods of preliminary storage at 80°F. were used, respectively (1) less than six hours, (2) three days, (3) five days, (4) seven days, and (5) ten days. The pods were afterwards stored for 15 days in the 53°F. chamber, after which the beans were put out for germination.

Table 37.

15 days storage at 53°F. to 54°F.

Time of pre-storage.	Percentage germination.	
	7 days	30 days
0 days	9	15
3 days	8	11
5 days	10	2
7 days	6	5
10 days	16	7

Summary and Discussion.

An account is given of the germination behaviour of fresh, ripe cocoa beans, and the way in which it is affected by storage both at tropical and sub-tropical temperatures. Points of importance are :—

(1) The cocoa bean is a non-resting seed, in that it is ready for germination when the fruit is ripe, and normally loses the capacity to germinate after a comparatively short period of storage, unless special precautions are taken. The deterioration seems to be due to three main causes :—desiccation, fungal decay, and "senescence", the last of which has not been investigated, but on general principles might be regarded as due to the gradual depletion of respiratory material. The first two, although essentially different, may practically be considered together, since measures designed to control one, *i.e.*, desiccation, are of no use unless steps are taken to control the other. To these may be added a fourth, when low temperature storage is considered, the reduction of viability due to some effect of chilling. In natural circumstances, *i.e.*, when overripe pods are left unpicked on the trees, the approach of senescence of the beans is marked by their spontaneous germination *in situ* provided the pod has escaped rotting or withering.

Similar germination was noticed in some of the stored pods when kept at 70°F. to 80°F. for eight to ten weeks. It was further noticed that these beans showed a poorer ultimate germination than initially ungerminated ones from the same pods. Although not examined experimentally, it is believed that there is a genetic factor influencing germination, and that certain trees yield beans from ripe pods which give a lower germination percentage than the normal 96 to 100 per cent. It is sometimes claimed, and supported by general plantation practice, that beans taken from pods not quite fully ripe, will always yield virtually complete germination, even with the 'difficult' trees mentioned. In these cases it may be that some shortcoming in the reserve food supply of the beans may start 'senescence' even during the ripening process. It is proposed to devote special study to this question of pod maturity as it affects the germination and storage capacities of the beans.

(2) The germination of fresh beans was not materially affected in the final extent, only in the initial rate, by germinating at temperatures ranging between 70°F. and 90°F. Neither was the final extent of germination much affected by soaking the beans in warm or tepid water before sowing, although the soaking showed an initial stimulus to germination rate. Tests of germination at different temperatures were not made on stored beans, but experience would suggest using a fairly high temperature, to avoid prolonging the incipient stages of germination, in which a high mortality frequently occurred. Soaking treatments with stored beans gave inconclusive results, in one trial soaking for one hour was injurious, but overnight soaking was beneficial, and in a second trial neither period had appreciable effect.

(3) The deterioration of the beans follows that of the pods, in which the factors of fungal decay and desiccation are of great importance. Thus in pods, not specially treated, after 20 days storage at tropical temperatures, rotting and drying-up of the pod walls is pronounced and advances rapidly from this time, accompanied by a rapid decline of germination rate. An effective control against desiccation was found in smearing pods with a thin layer of vaseline, but it is only effective in the absence of extensive fungal attack. No effective control against the development of dormant or incipient fungal infections was obtained, except that temperatures of 60°F. and lower, slowed down the rate of rotting. Special importance thus attaches to the selection of material, so that a sufficiently large sample of visibly unblemished pods should be collected. Those which start to decay in storage can be periodically discarded.

The effective lower lethal temperature lies between 45°F. and 50°F. No germination was ever observed at 45°F., but there was slight germination at 50°F. At 60°F. germination remains perfect for 40 to 50 days with no desiccation treatment, and would probably be

good for considerably longer with this treatment. Thus a sharp flection must occur in the temperature: germination curve between 50°F. and 60°F. This is supported by the results obtained at 53°F. to 54°F., a germination of 10 to 20 per cent. being obtained after 15 days storage. Unfortunately temperatures intermediate between 53°F. to 54°F. and 60°F. were not available so that the shape of the curve could only be inferred. It is thought that the curves shown in the graph (Fig. 8) represent fairly accurately the variation of germination with storage temperature for an *average* lot of cocoa pods stored for ten days, 20 days and 40 days respectively. The steep ascent of the curve represents the lessening of the lethal effect due to chilling, and the fall at temperatures above 60°F. is mainly due to fungal decay. The curves (except the ten days one) are based on germination figures of bulked samples of vaselined pods, with or without other antiseptic treatments, in which a fairly high proportion of fungal wastage occurred. In carefully selected individual pods in which this source of wastage does not occur, the curves follow the dotted lines, which it will be seen are of the same form as the curve for average pods for ten days storage, a period which is too short for fungi to penetrate into the bean cavity. Storage at 53°F. gives a low but not negligible germination, and inasmuch as fungal decay is very much slowed down at this temperature, it should be of advantage for storage when only blemished pods are available. It has an additional advantage that viability is rather helped than hindered by a delay, up to one week, between picking and cool storage. As the viability: temperature curve rises steeply at this temperature, advantage should be taken in storing the pods, of the rather higher temperature near the return ports of the air blast to the storage chamber.

(4) There seem to be two periods during the germination of a batch of stored beans, in which deaths frequently occur, first when the beans have just started to germinate and the radicles are a few millimetres long, and secondly some 10 or 12 days after sowing. The first is usual in beans which have been stored at low temperatures and the second in those which have been stored at the higher temperatures and in which fungal attack is present. In all the storage trials, those beans which survived to the four to five weeks count (effective germination), grew into normal seedlings when samples were planted out.

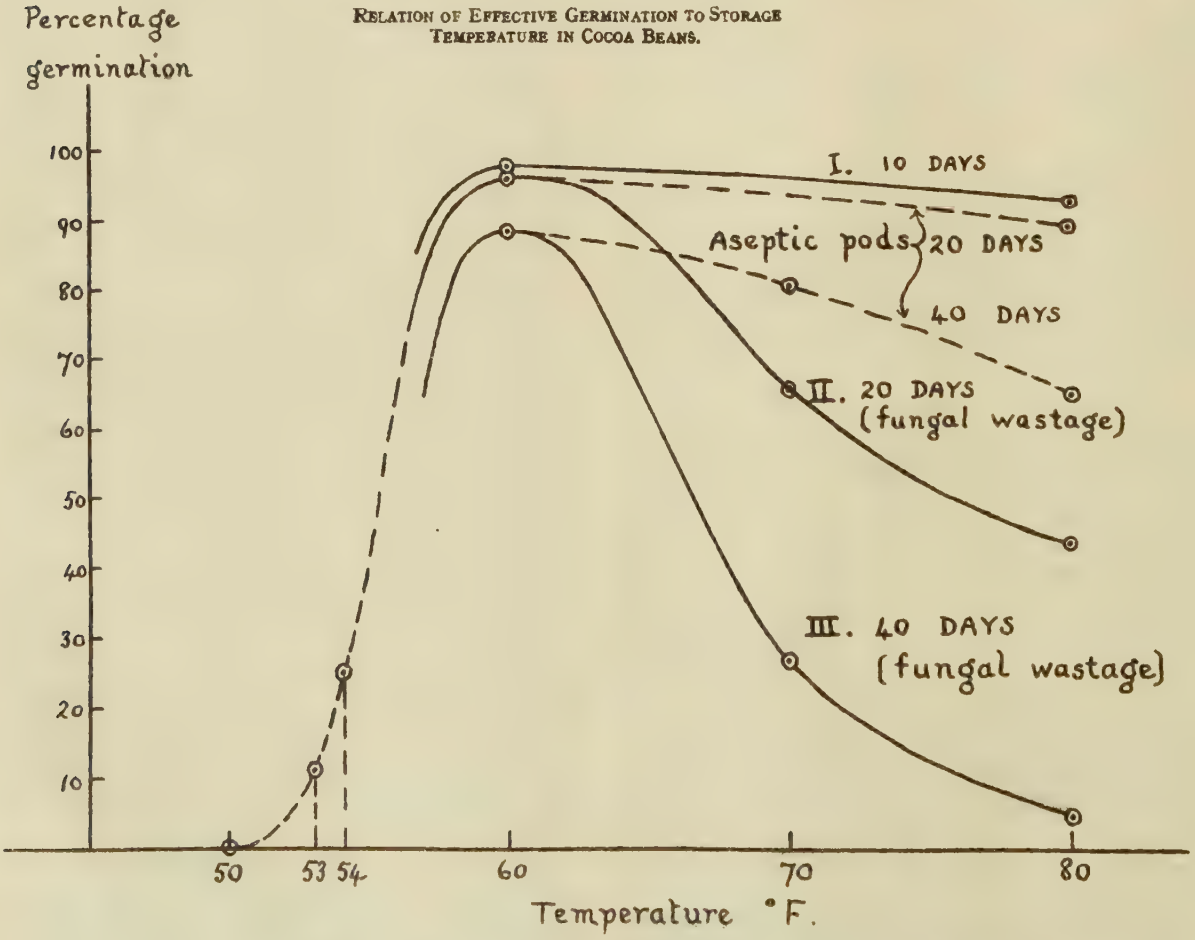

Fig. 8.—*Curve I represents the percentage effective germination after 10 days' storage, derived from general experimental data. Curves II and III (entire line portions) are from the experiments on antiseptic and anti-desiccation treated pods. The dotted line portions represent the germinations from the few completely non-infected pods in the same experiment. The dotted line portion of curve II at low temperatures is based on data from 15 days storage trials at 53°—54° F.*

MYCORRHIZA IN CACAO.

By E. E. Pyke.

1. Introduction.

Recent researches of the Chemical Section on the relation of the productivity of West Indian cacao soils to their organic matter content, have focussed attention on the physiological processes by which the plant is able to obtain nutriment from the humic layer. One factor, suspected, but not demonstrated, is the occurrence of a symbiotic fungus in the roots of cacao trees. The present paper gives a preliminary account of the search made for such a fungus, and of the occurrence and morphology of some forms which have been discovered in cacao roots in Trinidad plantations, and which on morphological grounds appear to have a symbiotic status. The writer considers that he is justified in using the term "mycorrhiza" for the fungal association found in many cacao rootlets, on account of the similarity of the organs present to those described by several other workers for the mycorrhizas of a number of plants. No physiological rôle is assumed for the fungal association in the use of the term "mycorrhiza" throughout this paper, except that the formation of 'arbuscules' and their subsequent degeneration and disappearance, is taken as evidence of a digestion process of the fungus by the root cells. It is sufficient to note that a regular fungal infection has been found in roots of cacao which agrees structurally with a certain type of accepted mycorrhiza, the so-called 'Phycomycete' type. In distinction to the other sorts of mycorrhiza, no critical work on the physiology of any of the phycomycete mycorrhizas has so far been published, no doubt owing to the difficulty of isolating the causal fungi and growing them in pure culture, so that the group is practically a morphological one.

It will be convenient to review briefly the history of mycorrhiza and to discuss its general significance. The word "mycorrhiza" means "fungus-root" and was first used by Frank in 1885, to describe a regular association formed between fungal hyphae and the roots of many species of temperate forest trees. Rayner defines mycorrhiza broadly as non-pathogenic infection by fungi in vascular plants and bryophytes. Although usually found only in the roots, the rhizomes, stems, and even the seeds are infected with mycorrhizal hyphae in some species. The distinction between mycorrhiza and feeble parasitism is not always obvious, but mycorrhiza may generally be distinguished by the following features:—(1) More regular occurrence than parasitic organisms; (2) formation of special organs, such as "arbuscules", "pelotons", and "hyphal mantles" which are constant for a given species. (3) Evidence of digestion of the fungus by cells of the host, and (4) (negative evidence) no signs of deleterious action upon the vigour of the host. (5) in some groups notably the Orchidaceae, and the conifers, there seems to be a virtual dependence upon infection and mycorrhiza formation, for the realisation of full vigour by the host.

Pfeffer in 1877 discovered a fungus of regular occurrence in the roots of orchids, and attributed to it a physiological function analogous to that of root hairs. Kamienski, in 1881, published a detailed description of the mycorrhiza of *Monotropa*, a non-chlorophyllous orchid, and later, in his work on seedlings of the same plant, he showed that the relation between the higher plant and the fungus was essentially different from parasitism. Again later (1886), he expressed the view that the roots of *Monotropa* derive nutrient from the surrounding fungus, although believing that the mycorrhizas found on the roots of Pine and Beech were parasitic. Frank (1885 and later), found that fungal infection was widespread in the roots of trees, and he extended the theory of beneficial symbiosis. Mycorrhiza was found only on soils containing abundant humus or plant detritus, and its luxuriant development was promoted or reduced according to the abundance or scarcity of these soil constituents. He believed that the root fungi supplied the roots with water and salts, and also with soluble organic matter abstracted from the humus. He obtained experimental evidence in support of this theory by growing seedlings in soils lacking humus, in sand, and in soils containing humus from sources other than the environment of the plants. In these experiments Frank found that the mycorrhiza tended to disappear when humus of the appropriate kind was lacking.

Since the discovery of mycorrhiza, great interest has centred about the nitrogen metabolism of mycorrhizal plants. Frank and Molisch showed that there existed a direct relation between nitrate deficiencies in the soil and in the plants, from which it was inferred that vascular plants are unable to convert either free nitrogen or nitrogen compounds into nitrates. Ebermayer in 1888 recorded the almost complete absence of nitrates from many woodland and moorland soils, and Frank in 1887 found in various tree species a complete absence of nitrates throughout the plant, except for sometimes minute traces in the absorbing roots. The conclusion was that mycorrhizal plants derived their nitrogen either directly from the humus, as ammonium salts or organic nitrogenous compounds, or from the humus *via* the fungi in the course of intracellular digestion. In this connection the conclusive demonstration of nitrogen-fixing ability by the fungus (*Phoma radicis* vars.) forming mycorrhiza in members of the Ericaceae is significant. (Ternetz.)

Non-chlorophyllous vascular plants, excluding parasites, with one possible exception always have mycorrhiza, and it seems very probable that in these plants the symbiotic fungi supply carbon compounds to their hosts. On this view it is incorrect to call such plants saprophytes, as they are parasitic upon the root fungi.

2.—Methods Employed in Collecting and Examining Material.

The top litter was scraped aside to expose the cacao rootlets. Fine and very fine rootlets, and larger roots were fixed in the field in separate phials, and a bunch of the fresh roots, together with a sample of the surrounding substratum was collected in a tin with a close fitting lid. Two alternative fixatives were used :—

1. Mercuric chloride .... 3 g.
   Glacial acetic acid .... 3 c.c.
   Water to .... 100 c.c. and

2. 6% soln. pot. dichromate } mixed at time of use in equal volumes
   10% soln. gl. acetic acid }

Both fixatives gave good results for routine work although for investigating the finer details of the "digestion" process a fixative with osmic acid would probably prove better.

In some cases freehand sections were made of fresh or fixed material, but generally the fineness of the mycorrhizal roots and the advantage of longitudinal sections made paraffin embedding and microtome sectioning necessary. Freehand sections of fresh or fixed material were stained with cotton blue in lacto-phenol. This formed a good temporary stain for both the internal and external hyphae. Microtome sections were cut longitudinally at 10μ and were stained with haematoxylin.

3.—Occurrence of Mycorrhiza.

Geographical.—Mycorrhizal roots were collected from estates in the Northern Range, the Central Range (district of Montserrat), and at Penal, in the extreme south of the island. The districts of Toco, Sangre Grande, and Rio Claro are still to be investigated. Samples were taken from the following estates in the Northern Range and all gave mycorrhizal roots :—River, Ortinola, Wardour, Verdant Vale, Torrecilla. Las Hermanas estate at San Rafael yielded some cases of mycorrhiza, but a rather high proportion of roots from this estate were free from infection. In the Montserrat district of the Central Range two samples were examined from different soils on San Juan estate, and both showed heavy mycorrhiza infection. In view of the accurate data available both of soil type and yield for this district, a detailed examination of roots from a number of selected sites is proposed, to find whether any correlation is evident between the density of mycorrhizal infection and yield, in the first instance, and the chemical and physical composition of the humic soil in the second.

Distribution in the Roots.—True mycorrhizas are formed only in the fine ultimate rootlets. These range in length from about 2 mm. up to 2 cms. but only in the shorter rootlets 2 mm. to 6 mm. in length, and from 0.2 mm. to 0.4 mm. in diameter is mycorrhiza frequent. The fungus seems always to be confined to the cortex, and to the zone of the rootlet which has root-hairs. The terminal growing tip, and the embryonic initials of lateral rootlets are always free from infection, as is the central vascular cylinder. In cacao, some rootlets assume the function of "runners" which extend the range of the root system. These rootlets, and their first order laterals are of much larger diameter than those of limited extension growth, and their cortices are always free from mycorrhiza. The incidence of mycorrhiza is very variable, sometimes practically every rootlet is infected, and in other samples the majority may be quite free from infection and only a few may form typical mycorrhiza. This is not correlated with the presence or absence of the superficial mycelium on the surface of the roots, or, it seems, with their vegetative condition. The possible bearing of soil factors on density of mycorrhiza development is being investigated.

4. Description of the Mycorrhiza.

A.—The Superficial Mycelium.—The mycorrhiza is of the type known as "endotrophic", that is, within the tissues of the root, as opposed to the "ectotrophic" type in which the fungus forms a dense weft or mantle over the surface of the root, with little or no penetration below the epidermis. Although forming an endotrophic mycorrhiza, the fungus has a well marked external phase upon the surface of the infected roots, but this does not approach the dense fungal investment of the ectotrophic type. However the mycorrhizal cacao roots always have this characteristic mycelium growing on the surface, which is clearly distinguishable from other chance epiphytic organisms frequently present, such as species of *Fusarium*, *Penicillium* and *Mucor*.

The hyphae of the endophyte are much branched and anastomosed, weaving among the root hairs from which they are sometimes difficult to distinguish in unstained preparations. In stained sections the hyphae show lightly staining granular contents, with deeply staining nuclei. The hyphal walls are extremely thin. The diagram (Fig. 9) illustrates this phase. The bases of the root hairs, the surface hyphae, and penetration by means of an appressorium (*see* following paragraph), are shown. Some of the hyphae are of larger diameter, and these seem to be longitudinal runners, or "stolons", which link up the separate regions of denser mycelial development. Lateral hyphae branch from these at angles of roughly 45° to 60°, not usually at right angles, and these hyphae frequently branch and anastomose, so that the surface of the root is invested in an open network. At junctions, the hyphae often swell into club-shaped vesicles, and where several hyphae unite, a large, often convoluted, multinucleate vesicle occurs.

B.—Penetration.—These vesicles or appressoria appear to play an important rôle in the entry of the fungus into the root. They are often very large and flattened, and from the undersides, which are adpressed to the root, the penetration hyphae develop. Figure 10, which is a later serial section through the same piece of root surface as figure 9, shows penetration into an epidermal cell (or sub-epidermal cell) with the production of a characteristic vermiform vesicle within the invaded cell.

C.—The Internal Mycelium.—The fungus, after penetrating the cuticle, forms in the epidermal cell a vesicle, from which another stout hypha grows into a cell below it and by radial growth through sucessive cells it reaches the deeper layers of the cortex (in the case illustrated only one or two layers intervened between the epidermis and the region of mycorrhiza formation) with practically no longitudinal or tangential penetration in these intervening layers. Thus the epidermis and the outer layers of the cortex may

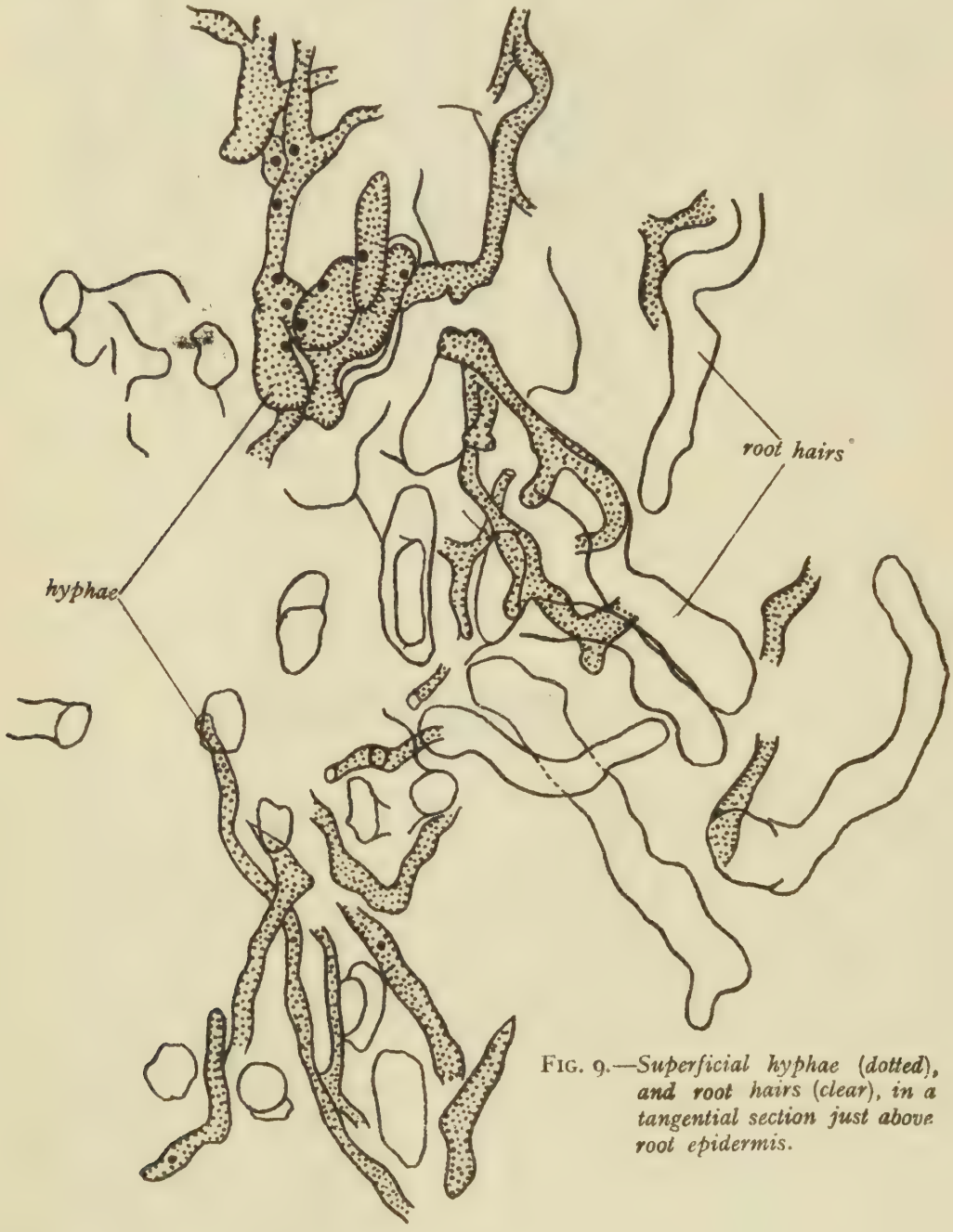

Fig. 9.—*Superficial hyphae (dotted), and root hairs (clear), in a tangential section just above root epidermis.*

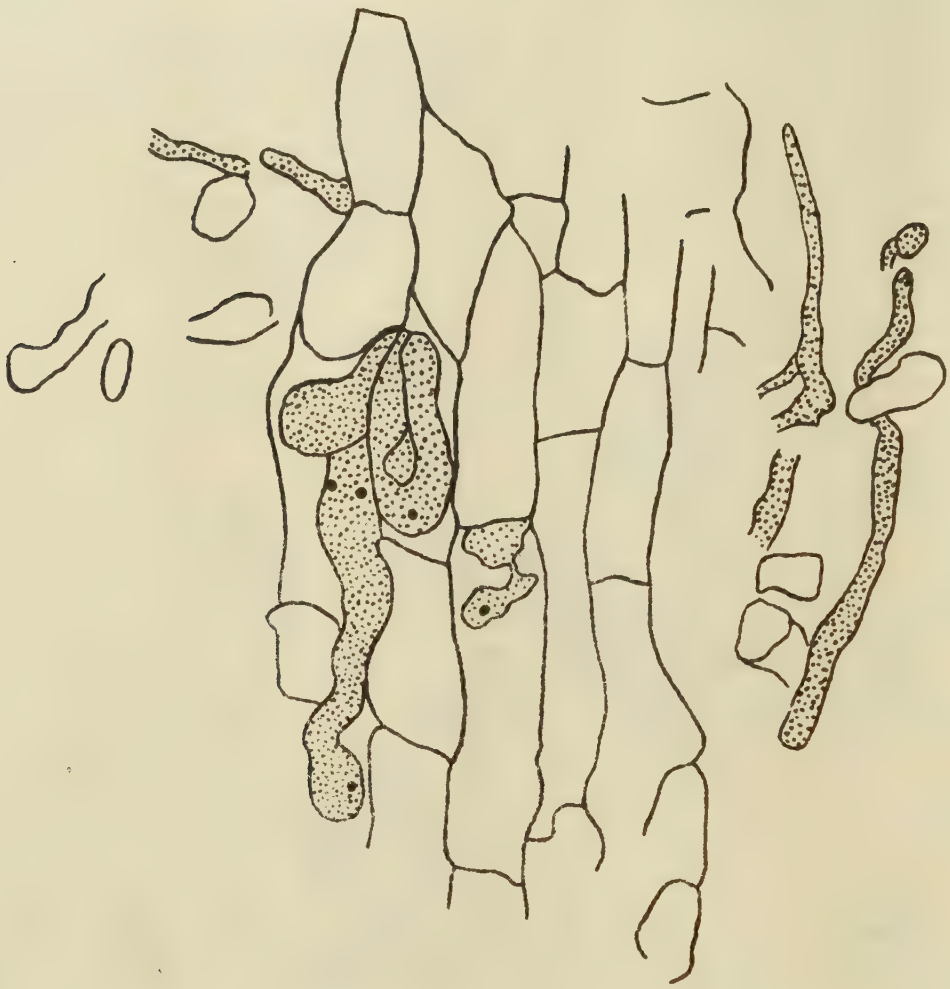

FIG. 10.—*Tangential section through epidermis showing vesciculate hyphae in epidermal cells.*

be entirely free from mycelium, except in the immediate region of penetration. At the mycorrhizal layer of the cortex however, the cells contain the characteristic curled vermiform hyphae, as shown in figure 11 having granular contents and densely staining nuclei, and showing considerable similarity to the vesicular stage of the external mycelium. The fungus in this stage appears to be entirely intracellular, and the hyphae are practically non-septate, septa being very rare. In the young rootlets under consideration, this "mycelial" stage of the mycorrhiza occupies only one cell-layer in the cortex, hence only in a longitudinal section tangential to this layer is the fungus abundantly visible. In other sections, including the radial longitudinal and the transverse, this mycelial stage is seen on edge or on end, so to speak, indeed in thin transverse sections sometimes little more than the cut ends of the hyphae are seen. Hence the mycelial stage forms a thin cylindrical mantle within the cortex. Except that the fungus is present, these cells appear normal, thus they are of the usual size and are mono-nucleate with normal nuclei. In parenthesis it may be mentioned that giant cells with more than one nucleus have been seen in mycorrhizal cacao roots, but these abnormal cells appear to be due to the attack of nematodes.

Immediately adjacent to the mycelial layer, and to the inside of it, is a layer of cells in which the fungus is abruptly transformed into a much branched, three dimensional maze of densely staining hyphae which completely fills each cell, and from which detailed structure is absent except in very thin and carefully prepared sections. It is seen that the hyphae are much finer than those of the mycelial layer, and are repeatedly branched, and somewhat anastomosed (fig. 11). Connection with the hyphae of the mycelial stage is apparent through the cell walls. These intracellular hyphal complexes are typical

FIG. 11.—*Tangential section through cortex showing mycelial and digestive phases of the mycorrhizal hyphae.*

" arbuscules " of Gallaud (8) present in the endotrophic mycorrhizas of nearly all plants except the Orchidaceae, in which the endophyte belongs to a different group of fungi.

In what are regarded as the earlier stages of digestion, the outlines of the individual hyphae and their nuclei can be distinctly seen, but in the subsequent stages the hyphae disintegrate and the host cells are filled with their fragmentary granular contents, which now stain deeper than in the mycelial phase. The hyphae undergo this process without forming the distinct spherical " sporangioles " characteristic of some mycorrhizas but a certain amount of knobbing or clumping has been observed in some of the material. This is regarded as a fixation artefact. Cells may exist in the digestive layers from which the hyphal contents have almost disappeared, a few granules and hyphal wisps alone remaining, indicating that absorption has taken place.

The presence of the fungus in all of the roots examined has been exclusively cortical, no penetration occurring in the endodermis or the vascular cylinder.

The foregoing description applies to the very fine roots, in which the cortex consists of only four or five layers of cells. The actual root from which the diagrams (Figs. 9 to 11.) were drawn was 0.18 mm. in diameter. The mycorrhiza present in these roots is of the simplest type, both in respect of the variety of organs formed and in the distribution within the root. Mycorrhiza has been examined in larger roots, which exhibits greater complexity both in form and in distribution. The larger the root infected, the greater appears to be the complexity of the mycorrhizal system, in that the mycelial and digestive phases respectively are no longer segregated to definite cell layers, and the digestive phase tends to occupy more cells in the cortex while the mycelial stage becomes partly intercellular. Septate hyphae also appear.

Other Infections.—Cacao roots are not infrequently infected with organisms other than those responsible for true mycorrhiza formation. Fungi, algae, nematodes, and mites all occur in certain localities. From the point of view of mycorrhiza study, other fungal infections are of some importance, particularly when it is required to form quantitative estimates of mycorrhiza incidence. Young cacao rootlets may be divided into four classes :—(1) those in which no fungal infection of any sort is visible, (2) those which show true mycorrhiza only, (3) those which are infected with fungal hyphae but which do not show the characteristics of mycorrhiza, *i.e.*, absence of arbuscule formation, and finally (4) those roots which have both mycorrhizal and other fungi. The first group contains the actively extending portions of the 'leaders' and main sub-laterals. These roots, although having primary cortices, are of fairly large diameter, say 0.5 to 1 mm., and do not develop mycorrhiza, even in a locality of dense infection. Where the incidence of mycorrhiza is light a fair proportion of the finer rootlets may also come in group 1, and together with those in group 3, make up the proportion of potential mycorrhizas which do not develop. Roots galled by nematodes, or cavitated by mites, frequently fall in groups 3 and 4.

The mycelial forms apart from true mycorrhiza often present are of several distinct types exhibiting differences in hyphal diameter, size, staining reaction, degree of septation, and growth habit. More than one form is often present in the same tissue, sometimes in the same cell. The detailed description of these forms is beyond the scope of this paper but their occurrence is mentioned so that it will be realised that the accurate diagnosis of mycorrhiza in cacao rootlets is not always easy. In roots from some estates particularly, plenty of 'borderline' infections occur in which apparently healthy roots show more or less fungal penetration of the cortex, but in which clear-cut mycorrhizal symptoms are absent or doubtful. In batches from other estates most of the roots may show a simple well defined mycorrhiza in which only the two forms of the mycelial phase, and the arbuscular phase are present. Similarly normal mycorrhizas may occur in the roots of individual samples from localities showing chiefly low incidence. The occurrence of non-mycorrhizal infections of low order parasitic nature in cacao rootlets is analogous to that of " pseudo-mycorrhiza " noted by Melin in the ectotrophic mycorrhizas of Pine and Spruce (*see* Rayner—Mycorrhiza), and by Rayner (Rayner, M.C.—Mycorrhiza in Relation to Forestry I. *Forestry* VIII : 2, 1934).

5. Possible Specific Significance of Mycorrhiza to Cacao.

It is only tentatively that any opinion can be advanced relative to the function of mycorrhiza in the cacao plant. The problem is awaiting experimental treatment, and until this yields data, it does not seem profitable to speculate upon the evidence supplied from investigations of plants growing under quite different conditions. The detailed analyses of Hardy and others (9) for a number of Trinidad and other West Indian cacao soils, show that 'good' and 'bad' soils respectively have certain well marked general properties. A " good " cacao soil generally shows : (*a*) A high pH value, indicating a slightly acid or neutral reaction, (*b*) a high C/N ratio, showing that plant detritus is present in a more or less undecomposed state, (*c*) a relatively high content of available phosphate, and (*d*) absence of

(8) Gallaud, I.—" Etudes sur les mycorhizes endotrophes." *Rev. Gen. Bot.* 17 (1905).

(9) Studies in West Indian Soils, Nos. 3, 4 and 7 dealing with cacao soils of Tobago, Grenada and the Montserrat District of Trinidad.

harmful chemical factors such as gypsum, which seems to exert a deleterious effect upon cacao when present in the soil in appreciable amounts, and in particular, appears to nullify the advantage of a high phosphate status. "Bad" cacao soils on the other hand usually have one or more of the following properties:—(*a*) a low pH value (acid reaction), (*b*) a low C/N ratio, and (*c*) a low amount of available phosphate. The mineral content of the cacao soils of Trinidad below the top six inches is generally low, and of the top six inches of soil the humic layer contains usually a major proportion of the three essential mineral elements, nitrogen, potassium and phosphorus, so that the availability to the plant of these elements as present in the humic layer must be of great importance. Hardy stresses the importance of the organic matter content:—"The organic matter content of West Indian cacao soils is closely related to their productivity. It is thought that the chief function of organic matter is as a supplier of mineral nutrients which are liberated and fixed when the organic matter decomposes. In support of this theory, it has been shown that the organic matter content of the soil is more closely related to its productivity in those areas where the use of artificial fertilisers is not a customary practice".

On this theory it will be realised that any mechanism which may be able to 'short-circuit' the process of transfer of mineral nutrients from the humic soil to the roots will be beneficial to the plants having command of it, and should give them an advantage in competition with plants which do not profit by the use of such a mechanism. It is tempting to think of mycorrhiza taking a part in this process. There is circumstantial evidence that a biotic factor may be operating. Thus the relation of productivity in cacao to soil organic matter is a correlation, in which the kind of organic matter, as expressed in the C/N ratio, rather than the absolute amount enters. Thus a high C/N ratio, from seven to ten, denoting relatively high amounts of undecomposed plant remains, is a more definite indicator of fertility than a low one, even though the total amount of organic matter, as determined by a carbon estimation, may be smaller in the first case than in the second. Experimental research on the cacao root systems at different levels of C/N ratio, both in the field and in pot cultures is needed in this connection.

Considering inorganic nutrients, the work of Hardy shows that nitrogen is rarely deficient in cacao soils. Cacao in Trinidad is almost invariably grown under a fairly close shade of leguminous trees (*Erythrina* spp.) whose roots, which possess numerous bacterial nodules, are extremely abundant in the top soil. Furthermore the fallen leaves of the shade trees form a considerable fraction of the raw material of the humic layer, so that the history of a well-tended cacao plantation is one of continuous nitrogenous mulching. Thus in contrast to the forest soils of temperate climates, the top layer of cacao soils generally gives a total nitrogen analysis of 0.2 to 0.6 per cent. and is rich in nitrates. Due however to the great rapidity of soil nitrification at tropical temperatures, the presence of nitrogen as ammonium compounds is difficult to detect. Thus unless cacao roots are unable directly to assimilate nitrates, the rôle of mycorrhiza as a nitrogen intermediary would not seem to be important. Quoting Hardy:—"No relationship has yet been found between the total nitrogen content and the yielding capacity of any of the cacao soils so far examined. The potash content of cacao soils, unlike nitrogen, varies within wide limits, but only when it is abnormally low does potash appear to be a limiting factor in the growth and cropping of cacao trees. The status of available phosphate in cacao soils however, in the absence of apparently harmful factors such as gypsum, is closely correlated with yield."

Thus of the chemical soil factors, a high C/N ratio and a high level of *available* phosphate are those most clearly connected with high yield. Now both these factors might be expected to exert a direct nutritional effect, amounting perhaps to control, upon the fungal flora of the soil, included in which is the assumed saprophytic phase of the mycorrhizal fungus. Most fungi obtain their energy requirements by breaking down carbon compounds, such as plant residues, but not all can carry the process down to CO_2, and many cannot use even much more complex compounds, depending upon the individual species or race of organism. It may be quite possible that the mycorrhiza organism can no longer develop as a soil saprophyte when the plant remains in the surface soil become highly humified. If infection of new roots must take place from the soil, as seems probable, mycorrhiza would tend to decrease in these circumstances. In relation to phosphate, most fungi need a much more generous supply for their active growth than do higher plants, so that if the mycorrhiza fungus follows this rule, it would be expected to be abundant in soils of high phosphate, and rarer in soils of lower phosphate, subject to the other factors for fungal growth not being limiting. It is interesting to note that there is evidence that some mycorrhizal fungi derive benefit from contact with roots by absorbing phosphatid substances which the living roots excrete in small amounts.

The question of the significance of mycorrhiza to the plantation cacao tree is now being investigated by examining root samples from different localities and seeking correlations with (*a*) cropping data (*b*) chemical constitution of the soil surrounding the surface roots, particularly in reference to phosphate status, reaction, and C/N ratio and (*c*) general ecological and cultivation conditions. The work is being done jointly with members of the Chemistry Department, and will form the basis of a later paper.

Summary.

(1) Roots from cacao trees growing in a number of localities in Trinidad were examined for mycorrhiza. An infection having definite symptoms of an endotrophic mycorrhiza was regularly found in most of the samples.

(2) The fungus was found only in young, fine rootlets, and in the zone of root-hair formation.

(3) In the simplest mycorrhizas, the fungus has an external phase consisting of hyphae which ramify on the root surface, and which form local denser aggregates. At these places are formed appressoria from which hyphae enter the epidermal cells, and by intracellular growth traverse one or more layers of the outer cortex. At a certain level in the cortex an extensive intracellular mycelium develops, consisting of coarse, non-septate hyphae, coiled in a worm-like manner within the lumina of the cells. These hyphae contain dense granular stainable protoplasm with abundant, deeply staining nuclei. In the adjacent deeper layers the mycelium appears, in sections of moderate thickness, completely to fill the lumina of the cells with densely staining, formless hyphal masses, but in thinner sections these resolve into branched, tree-like arbuscules. These represent the fungus in the earlier stages of digestion by the host; as digestion proceeds the arbuscules degenerate, lose definite outline, and have a less intense staining reaction.

(4) In rather larger roots the same characteristic phases, mycelial and digestive are present, but the distribution is more haphazard, neither phase being restricted to definite cell layers. In addition, septate hyphae appear in the mycelial phase, and some intercellular growth occurs.

(5) Hyphae which could not be traced to the mycorrhizal fungus were found in some roots. These appear to be weak parasites and can occur in both mycorrhizal and non-mycorrhizal roots.

(6) In all the roots examined mycorrhizal hyphae were confined to the cortex. The meristematic regions of terminal and lateral growing points were not penetrated.

(7) Attention is drawn to the possibility that mycorrhiza may prove to be a biotic factor influencing the mineral nutrition of the cacao tree. Circumstantial evidence bearing on this is being sought by sampling roots to estimate the percentage occurrence of mycorrhiza infection, together with chemical analyses of the surface soil immediately surrounding the roots.

B.—CHEMICAL AND ECOLOGICAL SECTION.

THE CHEMICAL PROGRAMME OF 1934.

By F. Hardy.

In scientific research, as in other affairs of life, one thing leads to another. Investigations of the cacao crop by chemical and physical methods were commenced in 1931, and the problems were approached from two main points of view, namely, (1) a systematic qualitative and quantitative study of the soil and atmospheric environments of two contrasted "good" and "bad" plots of established cacao, and (2) detailed soil surveys of representative cacao areas of determinable reputation and performance. These preliminary investigations revealed the chief characters of a good cacao soil; they also indicated the particular external factors that mainly confer high productivity or, contrarily, that seriously limit production.

The experimental methods and results were fully described by Mr. McDonald (the Chemical Officer) in the First and Second Annual Reports on Cacao Research, and certain aspects of the work were further described by him in the Third Annual Report, and by members of the College Chemistry Department, in special reports on the cacao soils of important districts, not only in Trinidad, but also in the neighbouring islands, Tobago and Grenada. Many of the findings may be applicable in cacao-growing regions occurring in other parts of the world, since they appear to be fundamental, as well as peculiar to the cacao crop.

Characters of Cacao Soils.

As an outcome of the preliminary investigations, the following discoveries were announced, (1) that the phosphate status of West Indian cacao soils is particularly important in conferring high fertility, (2) that available soil potash may be especially important in some instances, (3) that the nitrogen status is generally adequate in most of the cacao soils examined, (4) that the organic matter component, forming a natural surface cover and merging with the highly-organic upper shallow layer of soil, exhibits characteristic features (notably a high carbon-nitrogen ratio), which differentiate good cacao soils from bad.

These discoveries naturally led to field experiments, specifically designed to test the various indications suggested by the results of the soil investigations. The field experiments comprised a series of manurial trials, and trials of organic mulches. The first set of manurial experiments chiefly concerned the phosphate factor and, in certain cases, its interaction with potash. The effects of manuring were assessed, not only by records of yield increments, but also by chemical analysis of parts of the cacao tree. Interactions between phosphorus, potash and nitrogen were soon revealed, and their significance led to further general manurial trials, involving nitrogen, as well as phosphate and potash. Descriptions of the experimental procedures and statistical discussions of the results of these field experiments were presented by the Chemical Officer in the Second and Third Annual Reports; they also form the greater portion (Parts II and IV) of the present (Fourth) Report.

The Effect of Surface Litter and Mulches.

A striking feature of cacao cultivation, (and indeed of that of many other tropical orchard crops), is the beneficial effect on yield which usually follows the accumulation of leaf débris, or the deliberate addition of bulky organic mulches, such as cut scrub, tree prunings or hedge trimmings, spread over the soil surface. Various reasons have been suggested at different times to explain this action. In order to test some of the proferred explanations, preliminary mulching experiments were initiated in December, 1932, and early in 1933, on two contrasted kinds of cacao soil. They were designed to give information regarding the effects of mulching on moisture fluctuations, temperature changes, and nitrate status of the soil. The results obtained are presented in Part III of this report, and the conclusion is reached that the chief effect is a rapid increase in nitrate content of the shallow surface soil, due to marked acceleration of the processes of ammonification and nitrification by micro-organisms. This increased activity is maintained when further applications of mulch are made. It is apparently accounted for by the development of soil moisture conditions better suited to bacterial life and multiplication.

Harmful Soil Factors and Cacao Production.

Among the harmful factors that seem to depress cacao yields in Trinidad, the special importance of excessive concentrations of sulphate was suggested by soil analyses. Many of the cacao soils of Trinidad are derived from Miocene sedimentary clay formations that contain abundant crystalline gypsum (calcium sulphate). During soil genesis, this component has been more or less completely leached from the surface layers, but, in many instances, it has not been leached far enough below the rooting zone, or else the gypseous layers have been brought near to the surface by soil erosion, so that the cacao trees are bound to take up excessive amounts of sulphate. In this event, phosphate manuring confers no benefit; such soils appear definitely to be unsuited to successful cacao production, and their cultivation with other crops seems to be preferable.

The interactions between excessive sulphate and nutrient soil factors necessarily deserve special study, both in the field and in the laboratory, and such is now being afforded them.

The question is considered in the present report (Part VI), and some experimental results are therein presented. Iron compounds seem to be specifically involved in sulphate and phosphate relationships, and their rôle is being particularly investigated.

Nutrient Deficiency Symptoms.

Nutrient deficiencies often reveal themselves by characteristic symptoms affecting the appearance of the plant. Thus, a particular sort of leaf scorch affects fruit crops grown in England under conditions of soil potash deficiency, and nitrogen-deficient soils frequently impart an unhealthy pale-green colour to the leaves of crops supported by them. In order to ascertain whether similar symptoms affect cacao, water-culture and sand-culture experiments have been initiated. Some preliminary striking results of this phase of the cacao investigations are presented in the present report (Part V). Leaf-scorch in cacao can be induced by withholding potash in nutrient sand cultures, and leaf-scorch in the field has been observed where the soil is known by chemical tests to be deficient in available potash.

Scope of Present (Fourth) Report.

Part I (Soil Surveys) describes two more typical soils utilised for growing cacao in the Southern Plain of Trinidad. They are well developed in the Rio Claro District, where the cacao crop is still relatively young, but is said to be showing signs of diminished yields. One of the soil-types is derived from a highly calcareous marl. It represents an important class of cacao soil that has not before been examined. The results of the soil survey reveal the fact that this soil is nearly devoid of available phosphate. A phosphate manurial trial has shown that increases of yield of the order of 100 per cent. may be obtained by simpler phosphate treatment alone, and within one year of the time of application.

The other soil type is derived from a gypseous green clay, which is sometimes also calcareous. This soil closely resembles other gypseous types that occur in the Montserrat District of Trinidad previously described. Phosphate manuring confers no benefit.

Part II deals exclusively with the details of the more recent manurial experiments. It concludes with a list of manurial experiments on cacao now in being.

Part III concerns mulching trials already mentioned.

Part IV presents results of chemical leaf analysis which has proved to be a very valuable means of assessing the effects of manurial treatments. It has provided a useful index for gauging the physiological condition associated with high productivity in the cacao tree, and has furnished a procedure for diagnosing the nutrient requirements of the crop and the soil.

Part V deals briefly with the effects of different nutritional deficiencies on the growth and appearance of cacao.

Part VI consists of a discussion and some preliminary results of investigations designed to give further information regarding the peculiar relationships that obtain between phosphate, available iron compounds and sulphate in gypseous soils.

Ecological Studies.—The routine measurement of environmental conditions within two contrasted ("good" and "bad") cacao plots that were commenced at the very beginning of the Cacao Research Scheme in January, 1931, have been discontinued in 1934. The records were published and fully discussed in the first three Annual Reports. They showed the important effects of fluctuations in the moisture conditions of the atmosphere and the soil on vegetative-flushing, flowering, fruit-setting and shedding of flowers and young fruits. They indicated that the most suitable environment for the cacao tree is one in which moisture fluctuations are least, and demonstrated the value of overhead shade and wind-breaks in the cacao fields of Trinidad.

During the past year (1934), ecological measurements have been solely confined to other areas where more specific investigations are proceeding. Thus, records of relative humidity and evaporating ability of the air, and of fluctuations in soil moisture, have been obtained at the site of an extensive manurial experiment laid down by Officers of the Botanical Section. This experiment has provided material for correlating chemical composition of cacao leaves with yield, and for studying the effects of manures on flushing, flowering, fruit-setting and shedding. Other ecological records of a similar nature have been obtained at the sites of the mulching experiments.

It may eventually be possible to organise the simple routine measurement of certain meteorological factors affecting the growth of the cacao tree in representative districts in Trinidad where topography has variously affected rainfall and exposure to wind. By this means, an ecological survey of the cacao-growing regions of the Island may be rendered feasible.

PART I.

CACAO SOIL SURVEYS.

By F. Hardy, J. A. McDonald and G. Rodriguez.

(I) Introductory.

In order to gain additional information regarding the specific soil relationships of cacao, a study has been made of two distinctive soil-types, occurring in the Rio Claro District, South Trinidad. These soil-types have tentatively been called (*a*) Princes Town Marl Soil, and (*b*) Green Clay Soil, after the names given to their parent rocks in G. A. Waring's "Geology of Trinidad", 1926 (5). Neither of the soil-types is represented in the Central Range area previously surveyed (3, 4,), and no account of their features has heretofore been published.

The history of cacao planting in the Rio Claro District dates back about 40 years. The earlier crops were apparently satisfactory, and high yields were obtained. In recent years, however, yields have progressively diminished. The causes of the decline are not obvious, but it has frequently been suggested that the chief reason is soil deterioration. In order to gain information on this point, soil profile studies were accordingly undertaken at representative sites, lying mainly within the two soil regions. The methods of field and laboratory investigation were the same as those employed by us in previous cacao soil surveys (1, 2, 3), and need not again be described in this place.

(II) Description of the Parent Rocks.

(*a*) *Princes Town Marl* (Lower Middle Miocene).

Waring (5, p. 54) describes the parent material thus :—The unweathered marl, obtained at depths below 40 feet, is a dark-grey to green-grey, massive, highly calcareous deposit, which breaks into irregular fragments having conchoidal fracture but seldom exhibiting bedding. It contains over 35 per cent. of calcium carbonate, 40 per cent. of insoluble silicates, 8.5 per cent. of oxides of iron and aluminium, 0.4 per cent. of potash and soda, but only traces of phosphate and of sulphate. It consists chiefly of remains of foraminifera (mainly *Globigerina*). It weathers to a buff or white calcareous clay, yielding a jet-black surface soil.

(*b*) *Green Clay.* (Lower Middle Miocene).

Waring (5, p. 57) describes this material thus :—The Green Clay is a fairly well-bedded, pa'e yellowish-green-grey, foraminiferal claystone, occasionally containing, though not usually, thin calcareous bands. It is commonly highly gypseous. It weathers to a bright yellow-brown surface soil, but the colour may vary from rusty-brown, tawny yellow to yellowish-grey.

Stratigraphically, the Green Clay lies above, but is conformable with, the Princes Town Marl. The two formations, together with other distinctive Miocene sedimentary strata, have been folded along east to west axes (the Rio Claro Anticline), so that the various beds outcrop in more or less regular strips running roughly east-north-east to west-south-west right across the southern part of Trinidad. The area is a low-lying peneplain, modified by stream erosion into gently undulating country, bounded on the north by the limestone scarps of the Central Range, and merging southwards into the soft sandstone hills comprising the Southern Range. Westwards, the Southern Peneplain is mainly occupied by the sugar-cane lands of the Ste. Madeleine group of estates, where the friable black clay soils, derived from the Princes Town Marl, are noted for their high productivity, whilst the brown clay soils derived from the Green Clay, are not so fertile.

(III) Description of the Soil-types.

The results of the detailed field and laboratory examination of soil profile samples collected within the Rio Claro-Princes Town area in March, 1934, have been tabulated and discussed in a typescript report, privately circulated in July, 1934. Data referring to the two particular soil-types under consideration are presented for the surface 6-inch layer of soil in the accompanying table. The surface 6-inch layer of soil in cacao lands so far examined in Trinidad appears to contain most of the soil nutrients, and to comprise the main zone of feeding roots. For purposes of comparison, data for the chief cacao soil-types occurring in the Montserrat District of Trinidad (3, 4), are included at the bottom of the table.

(*a*) *Princes Town Marl Soil.*

The soil is a heavy clay, black in colour to depths of 6 to 20 inches (usually about 10 inches), and slightly acidic to nearly neutral (rarely alkaline) in the surface 6-inch layer. The depth at which free calcium carbonate occurs and at which the soil becomes distinctly alkaline, varies greatly from 0 to 60 inches (average, 24 inches), presumably owing to differences in the initial carbonate content of the parent marlstone, and to differences in the degrees of surface erosion and of downward leaching. The content of organic matter in the surface 6-inch layer is generally high to very high, and its depth of penetration is usually relatively great, the thickness of the humic layer containing over 0.5 per cent. of organic matter, varying between 16 and 30 inches (average, 21 inches). The carbon-nitrogen ratio for the topsoil is relatively high (7.3), indicating (according to our accepted standards) a cacao soil of high productivity. Available nutrients and rate of solution (electrical conductivity values) are very high in the topsoil, and available potash contents are also high. Available phosphate, on the contrary, is extremly low, this being the most striking feature of the soil-type within the area surveyed.

The black marl soils of the Rio Claro District appear to be capable, or to have been capable formerly, of producing good cacao crops of high yielding capacity. In two respects only would the black marl soils appear to differ markedly from the better cacao soils of the Montserrat District previously surveyed (3), namely, (*a*) in their very much lower contents of available phosphate, and (*b*), in the presence of a highly calcareous layer at varying levels below the soil surface. The average depth of this highly calcareous layer is generally less than two feet (24 inches), but the limited data so far collected suggest no obvious relationship between depth of calcareous layer and yielding capacity of the soil for cacao. Certain sites having the calcareous layer quite near to the surface apparently produce cacao crops giving large yields, even though the trees are old, whereas others, having deep-seated calcareous layers, are already showing signs of diminishing yield which cannot definitely be attributed to old age. The possible effect of the presence of a large amount of calcium carbonate in the soil lying within the rooting zone of the cacao tree cannot therefore be gauged from the field evidence so far obtained.

On the other hand, *the phosphate status of most of the black marl cacao soils within the Rio Claro area seems to be definitely unsatisfactory*. Provided the diminished yields are not due to general cultural neglect, the intensive use of suitable phosphatic manures may thus be expected to improve the productivity of such soils, and may confidently be recommended for trial. It may be noted, however, that two of the profiles (Nos. 11 and 12) examined in fields of black marl soil, where yields are said to have fallen off in recent years, showed a relatively high water-table, 5 to 6 feet below ground level, even in the middle of the dry season ; these sites may be waterlogged during rainy periods.

(*b*) *Green Clay Soil.*

The soil is a medium heavy clay or silty clay, exhibiting only a shallow (1½ to 3 inches) surface layer of black or sepia-coloured humic soil. The colour of the surface soil to depths varying between 6 and 18 inches (average, 12 inches) is bright yellow-brown or tawny. The subsoil below is conspicuously speckled salmon-pink on a yellow-grey or brownish-grey ground, down to depths varying between 18 and 46 inches (average, 39 inches). The reaction of the surface 6-inch layer is highly acidic (pH value between 6.0 and 5.0). The content of organic matter in this layer is medium high, but its depth of penetration is somewhat less than that in the black marl soil-type (*i.e.* about 17 inches). The carbon-nitrogen ratio for the topsoil is low (5.8) indicating a cacao soil of relatively low productivity. The subsoil below depths varying from 18 to about 28 inches (average, 24 inches), however, contains harmful amounts of sulphate, derived from included gypsum, conspicuous in the lower layers. Available nutrients and rate of solution are about half as great as in the black marl soil type, but available potash is high in amount. On the other hand, available phosphate is *extremely low*, even lower than in the last type of soil.

The Green Clay Soils of the Rio Claro District are much less productive than the black marl type, yielding only about half the weight of cacao for trees of similar age. This may perhaps be accounted for by their poor tilth and low permeability (due to an unsatisfactory lime status and lesser content of organic matter), and by their somewhat deficient nutrient status. Phosphate particularly seems to be lacking, though nitrogen may also be inadequate. The presence in the subsoil of harmful amounts of sulphate may adversely affect their productivity.

(IV) Summary and General Conclusions.

(1) The Princes Town Marl Soil and the Green Clay Soil, occurring widespread within the Rio Claro District of South Trinidad, differ widely in their physical and chemical attributes, and both are markedly different from the typical cacao soils of the Montserrat District of the Central Range region.

(2) The *Princes Town Marl Soil* might be described as a fertile soil, suitable for the growing of productive cacao crops, excepting that *its phosphate status is exceptionally poor.* This soil-type should benefit greatly by phosphate manuring. How far the nearness to the ground surface of subsoil containing large amounts of calcium carbonate affects the productivity of this soil, cannot be gauged from the limited data so far obtained.

(3) The *Green Clay Soil* is much less inherently fertile. In particular, the presence in the subsoil of gypsum in harmful amounts, appears markedly to depress its productivity for cacao. The content of available phosphate is also very low, but, in the presence of gypsum, phosphatic manures alone cannot be expected to effect increased productivity. In this respect, the Green Clay Soil resembles certain of the gypseous soils of the Montserrat District, notably the poorer kinds of Brasso Clay Soil previously described.

(4) Regarding the recorded decline in cacao production within the Rio Claro District, especial consideration should be given to the very low phosphate status of the soils. Assuming that adequate attention be paid to general sylvicultural treatment and soil management, especially drainage, it is believed that suitable manuring, particularly with phosphatic manures, should greatly improve cacao production in this area. The best manurial treatment, the magnitude of crop response, and the possible economic advantage to be gained from manuring, can only be learned by controlled manurial field experiments conducted at selected sites representative of the two main soil-types.

Addendum.

In Part II of this Report are described the results of two preliminary field experiments with superphosphate manure, laid down respectively on Princes Town Marl Soil and on Green Clay Soil within the Rio Claro District. In the first case (Princes Town Marl Soil), yield increases of the order of 100 per cent. were obtained with 1½ lb. and with 3 lb. of superphosphate per cacao tree. In the second case (Green Clay Soil), the yield response to superphosphate manure was *nil* (compare bad Brasso Clay Soil). This result may be attributed to the direct or indirect effect of the characteristic presence of gypsum in the Green Clay Soil-type.

(V) References.

(1) Studies in West Indian Soils (III); The Cacao Soils of Tobago; F. Hardy, C. G. Akhurst and G. Griffith; Supplement to *Tropical Agriculture*, February, 1931, pp. 1-23.

(2) Studies in West Indian Soils (IV); The Cacao Soils of Grenada; F. Hardy, J. A. McDonald and G. Rodriguez; *ibid.*, Dec., 1932, pp. 1-28.

(3) Studies in West Indian Soils (VII); The Cacao Soils of Trinidad (A) Montserrat District; J. A. McDonald, F. Hardy and G. Rodriguez; *ibid.*, Oct., 1933, pp. 1-50.

(4) Third Annual Report on Cacao Research, 1933. (B) Chemical and Ecological Section; J. A. McDonald; Imper. Coll. Trop. Agric., 1934, pp. 36-41.

(5) *The Geology of the Island of Trinidad, B.W.I.*, G. A. Waring. *The Johns Hopkins Press*, Baltimore, U.S.A., 1926.

RIO CLARO CACAO SOILS.

(Surface 6-inch layer)

	Yield; bags 1,000	Index of Texture.	Reaction.	Org. Matter	Nitrogen.	C/N Ratio	Avail. Nutr.	Rate of Soln.	Avail. P_2O_5	Avail. K_2O	Depth of carbonate or of gypsum layer.
		I.T.	pH.	%	%		mhos × 10-6		p.p.m.		(ins.)
(A) *Princes Town Marl Soil* (Calcareous subsoil).											Carbonate.
Poole (1)	11	60	6.6	4.50	0.35	7.2	161	129	19	169	24
Poole (2A)	Ban.	70	6.6	6.00	0.43	7.8	203	282	22	365	60
Poole (2B)	Ban.	64	7.0	2.22	0.20	6.0	238	166	30	318	6
Poole (5)	8	55	6.2	5.88	0.39	7.9	156	184	13	197	36
Gallera (7)	10	64	6.4	5.77	0.44	7.1	169	193	17	222	12
Gallera (8)	?	66	6.7	5.32	0.40	7.2	163	175	15	249	8
Rosario (9)	10	67	7.5	5.85	0.46	7.5	(431)	(423)	24	239	0
Rosario (9A)	10	66	6.8	3.79	0.29	7.5	213	161	16	176	12
Emilia (11)	9	67	6.5	5.92	0.39	7.7	174	158	18	226	36
Emilia (12)	6	63	6.2	3.04	0.30	(5.8)	123	68	11	177	48
Bajan (13)	12	48	6.4	2.85	0.28	(5.4)	120	136	6	168	24
Bajan (13A)	12	53	6.7	3.93	0.30	7.2	132	149	13	214	?
Means	10	62	6.6	4.59	0.35	7.3	168	164	17	227	24
(B) *Green Clay Soil* (Gypseous subsoil).											Gypsum.
Poole (6)	11	46	5.8	2.17	0.23	5.0	63	39	12	183	18
Poole (3)	7	36	4.9	3.71	0.30	7.2	69	41	15	144	48
Poole (4)	6	41	5.4	2.55	0.26	5.4	115	51	13	167	18
Poole (2)	Ban.	49	5.8	2.83	0.27	5.2	111	113	16	190	25
Rosario (10)	?	46	6.1	2.45	0.23	5.6	81	62	8	157	18
Bajan (14)	6	47	6.0	2.20	0.18	5.2	70	111	9	199	24
Forest (A)	—	51	5.3	2.25	0.23	5.5	47	13	19	178	18
Forest (B)	—	53	5.1	1.94	0.19	6.6	92	72	28	118	18
Forest (C)	—	55	5.2	3.82	0.35	6.1	120	135	(46)	184	33
Means	7	47	5.5	2.96	0.25	5.8	85	71	15	169	24
For Comparison: Montserrat District; Central Range.											
Chocolate Soil	—	43	7.2	4.5	0.34	7.2	216	175	343	309	—
Good Brasso Sand	—	22	6.6	2.5	0.25	6.3	132	131	53	455	—
Good Brasso Clay	—	37	6.9	3.3	0.28	7.3	215	166	71	145	—
Bad Brasso Sand	—	14	5.7	1.3	0.14	5.4	67	43	24	79	—
Bad Brasso Clay	—	40	6.6	2.9	0.28	5.8	191	121	71	151	—
Charuma Clay	—	44	5.5	3.8	0.30	6.5	116	84	29	112	—
Means: "Good"	—	40	6.9	4.0	0.33	7.1	216	183	149	254	—
Means: "Bad"	—	32	5.8	2.5	0.24	5.9	131	78	32	119	—

Deficiency of Phosphate (Avail. P_2O_5) *less than* 50 p.p.m.
Deficiency of Potash (Avail. K_2O) *less than* 150 p.p.m. (clay).
less than 100 p.p.m. (sand).

PART II.

MANURIAL EXPERIMENTS ON CACAO.

By J. A. McDonald.
(*Chemist for Cacao Research.*)

Series I.—Experiments with Phosphatic Manures.

Introduction.

This preliminary series of field experiments was initiated in 1932, and was designed to test one of the main findings of previous soil surveys of cacao-growing areas in the British West Indies, namely, a general deficiency in soil phosphate. The experiments aim specifically at estimating the response of the cacao tree to soluble phosphatic manures under different conditions of soil and atmospheric environment. In two of them, a sub-plot potash treatment was superimposed on the main plot phosphate treatments. Full details concerning the type of layout adopted and the methods of recording yields were given in the Third Annual Report on Cacao Research. Yield results for the first crop after manuring (1933) were also therein presented, and it was shown that marked increases in yield (of the order of 50 per cent.) were obtained by phosphate manuring at three different sites. Results obtained at three other sites during the first year were inconclusive, and discussion of the results was postponed until two years' records should be available.

In the following sections, results are presented for each of six different sites over a two-year period. All yield records have been subjected to statistical examination by means of Fisher's Analysis of Variance. The significance or otherwise of the results of each experiment have been tested by the usual "Z test", and all positive conclusions and recommendations are based on statistically significant results, having a minimum probability of twenty to one. From considerations of space, tables of statistics have been omitted from the text.

(I)—Las Hermanas Estate.

Soil Type.—Alluvial Clay, acidic in reaction.
Age of Trees.—Thirty to thirty-five years.
System of Replication.—One 4 x 4 Latin Square.
Size of Individual Plots.—18 trees.
Treatments :—1....Control
2....1 lb. superphosphate per tree.
3....2 lb. do.
4....3 lb. do.

Results :—The yield records for 1933 and 1934 are shown in Table I. The data were examined statistically, and the summarised results are presented in Table II.

Conclusions.

(1) Significant increases in yield have been obtained from all the different phosphate treatments at Las Hermanas.

(2) The highest yields (98.5 per cent. increase over the controls) have been obtained from plots receiving 3 lb. superphosphate per tree. These plots have given significantly higher yields than plots receiving smaller applications of 1 lb. per tree and 2 lb. per tree respectively.

(3) There is a significant difference in yield between the seasons, a much larger crop being picked in 1933 than in 1934. In common with most other cacao growing areas in Trinidad, the 1934 crop was very short in this district, owing to exceptionally wet weather.

Table I.

Las Hermanas Estate : Phosphate Experiment : Individual Plot Yields : Weight of Wet Cacao (lb.)

1933

A 80	D 72	B 55	C 37
C 70	A 114	D 68	B 39
B 50	C 81	A 69	D 47
D 60	B 31	C 68	A 49

1934

A 56	D 33	B 22	C 14
C 24	A 45	D 46	B 16
B 31	C 42	A 48	D 29
D 23	B 13	C 42	A 49

Size of Plot, 18 trees.
Treatments ; lb. Superphosphate per tree.
A—3 lb.
B—Control.
C—1 lb.
D—2 lb.

Table II.

Las Hermanas Estate : Phosphate Experiment.

Combined Yields for 1933 and 1934 : Weight of Wet Cacao.

Treatments Super-phosphate per tree.	Yields in lb. Wet Cacao. 1933	1934	Total.	Per cent. Increase over Control.
3 lb.	312	198	510	98.5
2 lb.	247	131	378	47.1
1 lb.	256	122	378	47.1
Control	175	82	257	—
Total for each Season	990	533	—	—

Each yield in Table II is the total of four plots. The Standard Error of the Difference between two totals is 39.8 lb. of wet cacao, and a difference of 97 lb. of wet cacao (equal to 37.7 per cent. increase in yield over the controls) may be regarded as significant, with a 20 : 1 probability.

Table III.

Las Hermanas.

COST OF SUPERPHOSPHATE COMPARED WITH VALUE OF INCREASED CROP.

SUPERPHOSPHATE.		INCREASE IN YIELD PER ACRE PER ANNUM.		VALUE OF INCREASE AT DIFFERENT PRICES.				
Application per tree.	Cost per acre per annum.	Wet Cacao lb.	Dry Cacao lb.	5 cents per lb.	6 cents per lb.	7 cents. per lb.	8 cents. per lb.	10 cents per lb.
3 lb.	$12.00	527	211	$10.55	$12.66	$14.77	$16.88	$21.10
2 lb.	8.00	252	101	4.04	6.06	7.07	8.08	10.10
1 lb.	4.00	252	101	4.04	6.06	7.07	8.08	10.10

Costs.

The approximate cost of the different superphosphate treatments compared with the value of the increased crop obtained are shown in Table III. In spite of the much larger yield obtained from the application of 3 lb. per tree, the greatest margin of profit is shown by plots receiving 1 lb. per tree, so long as the price of cacao remains below eight cents per lb.

Recommendations for Acidic Clay Soils.

The above results suggest that the best practical procedure for improving the phosphate status of cacao soils of this type would be an application of phosphatic manure equal to 3 lb. superphosphate per tree during the first year, followed by an annual application equal to 1 lb. per tree.

(II) Torrecilla Estate.

Soil Type :—Alluvial sand or sandy loam.
Age of Trees :—Fifty to sixty years.
System of Replication :—One 4×4 Latin Square.
Size of Individual Plots :—18 trees.
Treatments :—1....Control.
2....1 lb. superphosphate per tree.
3....2 lb. do.
4....3 lb. do.
5....½ plot ; 2 lb. potassium sulphate per tree.

Results :—The yield records for 1933 and 1934 are shown in Table IV. The data were examined statistically, and the results are summarised in Tables V, VI and VII.

Table IV.

Torrecilla Estate : Phosphate Experiment.

Plot Yields (1933), and ½ Plot Yields (1934) ; Weight of Wet Cacao (lb.)

1933

D 92	A 91	C 92	B 47
B 77	D 106	A 100	C 87
C 44	B 64	D 88	A 82
A 57	C 75	B 59	D 97

1934

58 D **82**	**67** A 95	54 C **61**	**41** B 52
41 B **65**	**100** D 55	67 A **44**	**34** C 56
54 C **65**	**69** B 65	61 D **71**	**78** A 51
22 A **42**	**37** C 40	28 B **45**	**28** D 63

Plot Yields (1933), and ½ Plot Yields (1934) ; No. of Pods.

1933

D 499	A 404	C 436	B 280
B 378	D 441	A 492	C 421
C 211	B 301	D 385	A 379
A 321	C 441	B 313	D 545

1934

238 D **442**	**324** A 496	281 C **317**	**208** B 291
208 B **425**	245 D 437	308 A **220**	147 C 289
274 C **335**	**336** B 345	298 D **319**	**386** A 217
93 A **225**	**237** C 190	145 B **246**	**135** D 363

Size of Plot, 18 trees.
Treatments ; lb. Superphosphate per tree.
A—3 lb.
B—Control.
C—1 lb. ½ Plot Potash Treatment,
D—2 lb. 2 lb. Potassium Sulphate **67**, &c.

Table V.

Torrecilla Estate : Phosphate Experiment.

COMBINED YIELDS FOR 1933 AND 1934 : WEIGHT OF WET CACAO.

Treatments Super-phosphate per tree.	YIELDS IN LB. WET CACAO.			Per cent. Increase over Control.
	1933.	1934.	Total.	
3 lb.	330	466	796	21.9
2 lb.	383	518	901	38.0
1 lb.	298	401	699	7.1
Control	247	406	653	—
Total for each Season	1,258	1,791	—	—

Each yield figure in Table V is the total of four plots. The Standard Error of the Difference between two totals is 44 lb. of wet cacao, and a difference of 107 lb. of wet cacao (equal to 16.4 per cent. increase in yield over the controls) may be regarded as significant, with a 20 : 1 probability.

Table VI.

Torrecilla Estate : Yields for 1934 : Weight of Wet Cacao from Potash Treated Half-Plots.

Half Plot Treatments.	MAIN PLOT TREATMENTS : SUPERPHOSPHATE LB. PER TREE. Yields in lb. Wet Cacao				Half Plot Total Yields.
	3 lb.	2 lb.	1 lb.	Con-trol.	
Potash	231	236	189	221	877
No Potash	235	282	212	185	914
Main Plot Total Yields	466	518	401	406	—

Each yield is the total of four half-plots. Analysis of Variance shows that differences between half-plot treatments are not significant, *i.e.*, there has been no response to Potash manuring at this site.

Table VII.

Torrecilla Estate : Phosphate Experiment.

NO. OF PODS PER LB. WET CACAO (SIZE OF BEAN) FOR 1933 AND 1934 COMBINED.

Treatments Super-phosphate per tree.	NO. OF PODS PER LB. WET CACAO.			Per cent. Increase over Control.
	1933.	1934.	Mean.	
3 lb.	4.90	4.88	4.89	9.2
2 lb.	4.87	4.85	4.86	9.9
1 lb.	5.05	5.15	5.10	4.7
Control	5.23	5.45	5.34	—
Mean for each Season	5.01	5.08	—	—

Each result is the mean of four plots. Analysis of Variance shows that differences in size of beans between treatments are not significant,

Conclusions.

(1) Significant increases in yield have been obtained from treatments of 3 lb. of superphosphate per tree, and 2 lb. per tree respectively.

(2) There is no significant increase in yield at this site from an application of 1 lb. superphosphate per tree.

(3) There has been no increase in yield due to potash manure (Table VI).

(4) There is an apparent difference in the size of cacao beans produced by the differently treated plots, *i.e.*, the plots receiving superphosphate manure have produced bigger beans than the control plots (Table VII). The tendency of the phosphate treated plots to produce bigger beans was noted in 1933, and the margin of difference was increased during 1934. This progressive increase in size is distinctly suggestive, but statistical analysis has shown that the results are not significant, and further evidence on this point must be awaited before definite conclusions can be put forward.

(5) There is a significant difference in yield between the two seasons, a very much larger crop being picked in 1934. This result is of some interest in connection with the incidence of Thrips attack in this locality. In 1933 there was a very severe infestation of Thrips in this area, but in 1934 there was only a slight attack. It is reasonable to suppose that the higher yields at this site in 1934 were due almost entirely to absence of Thrips damage, since, in most other areas in Trinidad, 1934 cacao yields were far below those of 1933. On this experimental block during 1933 (the year of heavy Thrips damage), there was a larger difference in yield between the control plots and the phosphate-treated plots than in 1934 (year of slight Thrips damage). This fact suggests that part of the benefit derived from the superphosphate manure has been to give to the cacao trees an increased resistance to Thrips attack.

Costs.

The approximate cost of the different super-phosphate treatments compared with the value of the increased crop obtained are shown in Table VIII. At this site an application of 2 lb. per tree is the only treatment which shows a margin of profit.

Recommendations for sandy and sandy-loam soils.

On light-textured soils of this type, optimum benefit may be expected from an application of phosphatic manure equal to 2 lb. superphosphate per tree. Applications of 3 lb. per tree appear to be definitely excessive, while no significant increase in yield could be demonstrated for an application of 1 lb. per tree.

Table VIII.

Torrecilla.

COST OF SUPERPHOSPHATE COMPARED WITH VALUE OF INCREASED CROP

SUPERPHOSPHATE.		INCREASE IN YIELD PER ACRE PER ANNUM.		VALUE OF INCREASE AT DIFFERENT PRICES.				
Application per tree.	Cost per acre per annum.	Wet Cacao lb.	Dry Cacao lb.	5 cents per lb.	6 cents per lb.	7 cents per lb.	8 cents per lb.	10 cents per lb.
3 lb.	$12.00	298	119	$ 5.95	$ 7.14	$ 8.33	$9.52	$11.90
2 lb.	8.00	516	206	10.30	12.36	14.42	16.48	20.60
1 lb.	4.00	96	38	1.90	2.28	2.66	3.04	3.80

(III)—River Estate.

Soil Type :—Alluvial silt.

Age of Trees :—Twenty-one years.

System of Replication :—One 3×3 Latin Square.

Size of Individual Plots :—16 trees.

Table IX.

River Estate : Phosphate Experiment.

Individual Plot Yields : No. of Pods.

1933			1934		
A	B	C	A	B	C
400	189	225	242	171	281
B	C	A	B	C	A
180	411	227	226	388	525
C	A	B	C	A	B
289	363	127	328	355	243

Size of Plot, 16 trees.

Treatments :—A—3 lb. Superphosphate per tree.
B—Control.
C—1½ lb. Superphosphate per tree.

Table X.

River Estate : Phosphate Experiment.

COMBINED YIELDS FOR 1933 AND 1934 : PODS PER TREE.

Treatments: Super-phosphate per tree.	YIELDS IN PODS PER TREE.			Per cent. Increase over Control.
	1933.	1934.	Total.	
3 lb.	21	23	44	91
1½ lb.	19	21	40	74
Control	10	13	23	—
Mean for each Season	17	19	—	—

Each yield given in Table X is the mean of three plots. The Standard Error of the Difference between two totals is three pods per tree, and a difference of 12 pods per tree (equal to 52 per cent. increase in yield over the controls) may be regarded as significant with a 20 : 1 probability.

Treatments :—1....Control.
2....1½ lb. superphosphate per tree.
3....3 lb. do.

Results :—The yield records for 1933 and 1934 are shown in Table IX. The records were examined statistically, and the results are summarised in Table X.

Conclusions.

(1) Significant increases in yield have been obtained from applications of 3 lb. superphosphate per tree, and from 1½ lb. per tree respectively.

(2) Previous records of yield, presented in the Third Annual Report, show that the plots receiving applications of superphosphate manure have a considerably higher *natural yield* than the control plots. For this reason, this experiment is not altogether satisfactory. However, when the yields for the experimental years are corrected by means of the previous records, significant increases in yield of 34.8 per cent. and 40.98 per cent. are still obtained from the superphosphate treatments of 3 lb. per tree and 1½ lb. per tree respectively.

Costs.

Yield records for this experiment were taken as number of pods only, and it would be of no value to draw up a detailed table of costs, since this would involve conversion of yields in number of pods to weight of dry cacao by an arbitrary factor. Comparison with the results recorded in Tables III and VIII indicates that a margin of profit is shown by the increased yield due to an application of 1½ lb. of superphosphate per tree.

Recommendations for fine-textured silt soils.

The best practical procedure for improving the phosphate status of soils of this type would be an application of phosphatic manure equal to 1½ lb. per tree. The general results obtained from the field experiments at Sites II and III show that phosphate manuring should be carried out with caution on sandy or silty soils, such as occur in the valleys of the Northern Range of Trinidad. Phosphate manures should only be applied to such soils when chemical analysis has indicated deficiency of available phosphate, and then only

in moderate applications equal to a maximum of 2 lb. per tree. Soil analysis has shown that many sandy or silty soils of this type are characteristically deficient in *potash* rather than phosphate, and the results of a field manurial experiment presented by F. J. Pound in the Botanical Section of this report have shown very marked increases in yield due to potash manure.

(IV)—Rio Claro (1).

Soil Type:—Black Calcareous Clay ; Princes Town Marl.

Age of Trees :—Twenty to twenty-five years.

System of Replication :—One 3×3 Latin Square.

Size of Individual Plots :—16 trees.

Treatments :—1....Control.
2....1½ lb. superphosphate per tree.
3....3 lb. do.

Results :—The yield records for 1933 and 1934 are shown in Table XI. The records were examined statistically, and the results are summarised in Table XII.

Table XI.

Rio Claro 1 : Phosphate Experiment.

Individual Plot Yields : No. of Pods.

1933		
B	C	A
663	405	246
A	B	C
322	506	393
C	A	B
349	194	335

1934		
B	C	A
441	425	49
A	B	C
226	354	340
C	A	B
267	62	151

Size of Plot, 16 trees.
Treatments :—A—Control.
B—1½ lb. Superphosphate per tree.
C—3 lb. do.

Table XII.

Rio Claro 1 : Phosphate Experiment.

COMBINED YIELDS FOR 1933 AND 1934. PODS PER TREE.

Treatments Super-phosphate per tree.	Yields in Pods per Tree. 1933.	1934.	Total.	Per cent. Increase over Control.
3 lb	24	22	46	100
1½ lb.	31	20	51	121
Control	16	7	23	—
Average for each Season	24	16	—	—

Each yield is the mean of three plots. The Standard Error of the Difference between two totals is three pods per tree, and a difference of 13 pods per tree (equal to a 56.5 per cent. increase in yield over the controls) may be regarded as significant with a 20 : 1 probability.

Conclusions.

Significant increases in yield (more than 100 per cent. increase over the controls) have been obtained from both superphosphate treatments.

Costs.

Yield records are available as number of pods only, therefore no detailed table of costs is presented. Comparison with Tables III and VIII indicates that the value of the increased yield would be more than the cost of the manure for both treatments, the biggest margin of profit being shown by an application of 1½ lb. per tree.

Recommendations for black calcareous-clay soils.

The best practical procedure for improving the phosphate status of soils of this type would be an application of phosphatic manure equal to 2 lb. superphosphate per tree during the first year, followed by an annual application of 1 to 1½ lb. per tree.

(V)—Rio Claro (2).

Soil Type :—Gypseous clay ; Green Clay.

Age of Trees :—Twenty to twenty-five years.

System of Replication:—Two 3×3 Latin Square.

Size of Individual Plots :—16 trees.

Treatments :—1....Control.
2....1½ lb. superphosphate per tree.
3....3 lb. do.

Results :—The yield records for 1933 and 1934 are shown in Table XIII. The records were examined statistically, and the results are summarised in Table XIV.

Table XIII.

Rio Claro 2 : Phosphate Experiment.

Individual Plot Yields : No. of Pods.

1933		
B	C	A
304	351	324
A	B	C
484	308	291
C	A	B
311	156	215
B	C	A
185	114	242
A	B	C
244	247	184
C	A	B
362	323	221

1934		
B	C	A
222	236	221
A	B	C
206	202	126
C	A	B
99	39	65
B	C	A
139	97	94
A	B	C
113	203	52
C	A	B
170	165	102

Size of Plot, 16 trees.
Treatments :—A—Control.
B—1½ lb. Superphosphate per tree.
C—3 lb. do.

Table XIV.

Rio Claro II: Phosphate Experiment.

COMBINED YIELDS FOR 1933 AND 1934: PODS PER TREE.

Treatments Superphosphate per tree.	YIELD IN PODS PER TREE.		
	1933.	1934.	Total.
3 lb.	17	8	25
1½ lb.	15	10	25
Control	18	9	27
Average for each Season	17	9	—

Each yield is the mean of six plots.

Conclusions.

At this site, the cacao trees have shown no response to phosphate manure.

Recommendations for gypseous clay soils.

From the results of this experiment, it would appear that no benefit is derived from manuring cacao soils of this type with superphosphate, even though chemical tests have shown a marked deficiency of available soil phosphate. The significance of these results in connection with the gypsum content of the subsoil layers will be discussed in a later section.

(VI)—Santa Serviera Estate.

Soil Type:—Gypseous Clay; Brasso Clay.
Age of Trees:—Forty years, approximately.
System of Replication:—One 4×4 Latin Square.
Size of Individual Plots:—18 trees.
Treatments:—1....Control.
2....1 lb. superphosphate per tree.
3....2 lb. do.
4....3 lb. do.
5....½ plot; 2 lb. potassium sulphate per tree.

Results:—The yield records for 1933 and 1934 are shown in Table XV. The records were examined statistically, and the results are summarised in Tables XVI and XVII.

Conclusions.

(1) The general yield of cacao at this site is very low. During the second year after the application of the manures, there was an apparent increase in yield for plots treated with superphosphate. The Analysis of Variance has shown, however, that these increases are not significant, and must therefore be regarded as chance variations between the plots.

(2) There is no significant increase in yield due to potassium sulphate manure.

Table XV.

Santa Serviera Estate: Phosphate Experiment.

Plot Yields (1933), and ½ plot Yields (1934): No. of Pods.

1933

C 80	B 28	A 36	D 112
B 31	C 76	D 102	A 86
D 107	A 222	C 221	B 160
A 158	D 51	B 208	C 108

1934

14 C **49**	**57** B 4	25 A **65**	**78** D 23
8 B **28**	**8** C 17	54 D **39**	**24** A 39
39 D **67**	**144** A 53	73 C **92**	**116** B 62
63 A **30**	**44** D 62	139 B **102**	**24** C 108

Size of Plot, 18 trees.
Treatments: lb. Superphosphate per tree.
A—3 lb. B—1 lb.
C—Control D—2 lb.
Half-plot—2 lb. Potassium Sulphate per tree. **57**, &c.

Table XVI.

Santa Serviera Estate: Phosphate Experiment.

COMBINED YIELDS FOR 1933 AND 1934: PODS PER TREE.

Treatments Super-phosphate per tree.	YIELDS IN PODS PER TREE.			Per cent. Increase over Control.
	1933.	1934.	Total.	
3 lb.	7	6	13	8.3
2 lb.	5	6	11	—
1 lb.	6	7	13	8.3
Control	7	5	12	—
Average for each Season	6	6	—	—

Each yield is the mean of four plots. Analysis of Variance shows that differences in yield between treatments are not significant.

Table XVII.

Santa Serviera Estate.

YIELDS FOR 1934: NO. OF PODS PER TREE FROM POTASH TREATED HALF-PLOTS.

Half-Plot Treatments	MAIN PLOT TREATMENTS: SUPERPHOSPHATE LB. PER TREE. Yields in Pods per Tree.				Half-Plot Mean Yields.
	3 lb.	2 lb.	1 lb.	Control.	
Potash	7.0	6.5	8.4	4.7	6.65
No Potash	5.0	5.5	5.6	5.3	5.35
Main Plot Mean Yields	6.0	6.0	7.0	5.0	—

Each yield is the mean of four half-plots. Analysis of Variance shows that differences between half-plot treatments are not significant, *i.e.*, there has been no response to potash manuring at this site.

Recommendations.

The results of this experiment serve to confirm the finding that superphosphate manure may be of little or no benefit to cacao trees growing on gypseous clay soils. Further experiments are needed to discover whether soils of this type will respond to types of phosphatic fertilisers other than superphosphate.

General Conclusions.

The superphosphate manurial experiments have conclusively demonstrated that the yield of mature cacao trees on many different types of soil in Trinidad, *can be very greatly increased by the use of phosphatic manures alone.* The increases in yield have varied from thirty per cent. on a light-textured sandy loam, to over one hundred per cent. on a fairly heavy-textured black calcareous clay. The results provide striking confirmation of one of the main findings of previous cacao soil surveys, and serve to demonstrate the great practical value of soil survey work, such as has been carried out during recent years by the College Department of Chemistry over a wide area in the British West Indies.

The importance of previous knowledge of soil conditions is obvious, since it not only ensures that field experiments shall provide information on problems of widespread rather than local importance, but it also ensures that the sites of the experiments are so selected that the results obtained are characteristic of a wide area on which cacao is grown. Without an accurate knowledge of soil conditions down to a depth of six feet (such as is provided by the profile method of examination), it would not have been possible accurately to interpret the results of phosphate experiments on gypseous soils. On such soils, there has been no response to phosphate manuring, and, in forming conclusions concerning the phosphate nutrition of the cacao crop, gypseous soils must be regarded as exceptional, and must receive separate consideration.

The cacao soil surveys showed that phosphate deficiency in cacao soils is a feature of widespread and fundamental importance, not only in Trinidad, but also in other cacao growing areas in the British West Indies. The very striking results now obtained in this series of manurial experiments are therefore capable of wide application in those areas in which soil phosphate deficiency has been indicated by soil analysis.

(A)—Non-Gypseous Soils.

The experiments have covered a wide range of different soil types, as is shown in Table XVIII.

Table XVIII.

Sites of Series I Phosphate Manurial Experiments: Increases in Yield Compared with Composition of Top 6-inch Layer of Soil and Gypsum ($CaSO_4$) Content at 3 ft. to 4 ft. Depth.

Soil Type and Locality.	Sand.	Index of Texture.	Reaction.		O.M.	N.	C/N Ratio.	Avail. K_2O	Avail. P_2O_5	$CaSO_4$ at 3 ft. to 4 ft. (Conductivity Value).	Average Per cent. Increased Yield due to Phosphate manure.
			Norm.	Exch.				p.p.m.			
	%	(I.T.)	(pH).	(pH).	%	%					
Non-Gypseous Soils.											
(1) *Las Hermanas* Clay Alluvium	9.4	47	6.2	5.4	2.99	0.27	6.0	139	29	92	64.2
(2) *Torrecilla.* Sandy Alluvium	53.7	23	6.7	6.2	1.80	0.16	6.5	150	21	40	22.3
(3) *River Estate* Alluvial Fine Silt	4.6	35	6.0	5.3	2.39	0.19	7.3	56	15	—	82.0
(4) *Rio Claro I.* Calcareous Clay....	20.5	48	6.5	5.8	2.82	0.28	5.4	168	6	(521)*	110.0
Gypseous Soils.											
(5) *Rio Claro II.* Gypseous Clay	11.1	47	6.0	5.0	1.85	0.18	5.8	199	9	2592	Nil.
(6) *Sta. Serviera.* Gypseous Clay	13.5	40	6.3	5.5	2.14	0.23	5.4	140	79	1371	5.2

* Due to excessive calcium carbonate and not to gypsum.

In addition to demonstrating the general value of phosphate manuring, the experiments have provided information on the optimum quantities of manure for each particular soil type. When the cost of the manure is compared with the value of the increased crop, it is evident that the optimum dressing for all soil types is 2 lb. superphosphate per tree (600 lb. per acre). In the case of clay soils with a markedly acidic reaction, such as the Las Hermanas site, further large increases may be obtained from applications of 3 lb. per tree (900 lb. per acre). With the price of cacao at its present low level, however, the additional increase does not compensate for the increased cost of the manure.

Markedly acidic clay soils are known to have a high absorbing ability for phosphate, whereby added phosphate is fixed in the surface layers of soil in a form unavailable to the plant. This may explain why the heaviest application of 3 lb. superphosphate per tree gave the highest yield at Las Hermanas.

Neutral or faintly acidic clay soils, such as the soil type of Rio Claro I, may possess a smaller fixing ability for added phosphate, and, for such soils, applications of 2 lb. per tree appear to be adequate.

For the sandy soil at Torrecilla, the highest yield was given by an application of 2 lb. of superphosphate per tree, and applications of 3 lb. per tree appear to be definitely excessive on this type of soil.

These results justify the general recommendation that, in order to improve the phosphate status of low-yielding cacao soils, phosphatic manures should be applied at a rate equal to 2 lb. superphosphate per tree. This amount may have to be varied slightly, depending on the physical and chemical characteristics of the soil.

(B)—Gypseous Soils.

At Sites V and VI, the cacao trees have shown no response to phosphate manures applied over a period of two years. The soils of both these sites have one common characteristic which sharply differentiates them from the soils at Sites I to IV, *i.e.*, they possess a high content of soluble calcium sulphate (gypsum) at a comparatively shallow depth in the profile. The gypsum accumulations at Sites V and VI are shown in Table XVIII by the abnormally high electrical conductivity values of samples taken from the 3 ft. to 4 ft. layer in the soil profile.

The above results suggest that cacao soils of this type, showing a high gypsum content in the sub-soil layers, may fail to respond to phosphatic manures, even though chemical analyses and pot tests have shown a marked deficiency of available soil phosphate.

Previous results obtained in a survey of cacao soils occurring within the Central Range area of Trinidad, support this finding. Within the Brasso Clay soil belt (on which Experiment VI is located), good and bad cacao areas are sharply differentiated by the gypsum content of the sub-soil layers. The good and bad areas within this soil type are otherwise similar in their physical and chemical characteristics. The gypsum content of the soil would appear therefore to be a definite factor which produces harmful effects on the growth and yield of cacao. The results obtained in Experiments V and VI suggest that this harmful effect is connected with the utilisation of phosphate by the cacao tree, trees growing on gypseous soils being unable to make proper use of naturally-occurring soil phosphate or phosphate added in artificial manures. The phosphate-gypsum problem in cacao soils is the subject of a special note in a following section of this report. In this note, the suggestion is put forward that phosphate may have a depressing effect on growth and yield in gypseous soils, in that it interferes with the availability and utilisation of *iron* by the cacao tree. Experiments have been designed, and are now being carried out, to test this hypothesis.

Future Work.

Field manurial experiments form an integral part of the investigations of the Chemical Section of Cacao Research, and aim at providing fundamental information concerning the correct mineral nutrition of the cacao tree. Results of the field experiments are always considered together with results of other chemical investigations on the composition of the soil and of the cacao tree itself. By these means, a more exact interpretation, and a more widespread application of the results, are rendered possible. Each experiment is designed to test a particular problem suggested by the results of previous investigations. The following additional series of field experiments have already been laid down.

(A)—Series II. Phosphate Experiments.

Series I phosphate experiments proved the general benefit of phosphate manuring to the cacao crop. The experiments of Series II are laid down on soil where phosphate manuring has proved to be of benefit. They are designed to test the comparative value of different types of phosphatic manures, such as superphosphate, double superphosphate, raw rock phosphate, and ammonium phosphate.

(B)—General Nitrogen, Phosphate and Potash Experiments.

These experiments have been laid down at River Estate, the Trinidad Government Cacao Experiment Station in the Diego Martin Valley. They are being carried out with the co-operation of the Trinidad Department of Agriculture, to whom we are greatly indebted for supervising the collection of all yield records. Chemical tests have shown that the soil of this area is markedly deficient in potash, as well as phosphate (Table XVIII). Previous soil surveys have shown no marked potash deficiency in the majority of West Indian cacao soils, and in Series I experiments, no response in yield from potash manuring could be demonstrated. The sandy types of soil on which cacao is grown in the Diego Martin Valley, and in many other valleys of the Northern Range in Trinidad, show a characteristic low potash content, and positive results may be expected from experiments involving the use of potash manures and their interaction with phosphate and nitrogen.

One of these experiments consists of two 5×5 Latin Squares, one square being laid out on a heavily-shaded area, and the other on an area carrying no overhead shade. It should here be possible therefore to study the effects of nitrogen, phosphate and potash manures on cacao growing under no shade conditions (such as exist in

Grenada and other parts of the world) as contrasted with the Trinidad practice of heavy shading. Many authorities in Trinidad have contended that the presence of shade trees in cacao cultivations seriously affects the possible benefit of applications of artificial manures, since the Immortel shade trees are voracious feeders, and take up a large proportion of the added manure. In addition to providing a solution to this problem, the experiment is designed to give information concerning the possibly different physiological requirements of the cacao tree for nitrogen and potash under shaded and unshaded conditions respectively. The possibility that different levels of nitrogen and potassium nutrition might be necessary under different degrees of light exposure was fully discussed in the Third Annual Report on Cacao Research.

(C)—Mulching Experiment.

Soil surveys have shown that high yielding capacity of cacao soils is usually associated with a wide carbon/nitrogen ratio of the soil organic matter. Accordingly, the application of vegetable mulch to low-yielding soils, as a means of widening the C/N ratio, was recommended. On a naturally highly fertile soil, increased growth of the cacao tree ought normally to occur, and a comparatively larger amount of natural mulch should be returned to the soil in the form of fallen leaves, &c. The possibility that wide C/N ratios for high-yielding soils may merely furnish an index of this increased growth has not been disregarded; nevertheless differences in the C/N ratio of good and bad cacao soils have been so marked and so consistent as to justify a special field experiment to test the effect of a wide-ratio vegetable mulch on the yield of cacao.

The mulching experiment consists of one 4 × 4 Latin Square, on which previous records of yield are available. The main plot treatments consist of interactions between nitrogen, phosphate and potash, while, the mulch is applied in strips as a half-plot treatment, at the rate of twenty tons dry mulch per acre.

(D)—Gypsum (Calcium Sulphate) Experiment.

Series I phosphate experiments showed that cacao trees failed to respond to phosphate manuring on soils having a high content of soluble calcium sulphate in the sub-soil layers, even though chemical tests and pot tests had shown such soils to be deficient in available phosphate. This result is the more surprising in view of the very large increases in yield obtained from phosphate manuring on non-gypseous soils.

In Trinidad, large areas of cacao are grown on soils derived from gypseous clay formations, in which high concentration of gypsum may occur at comparatively shallow depths in the profile. In all the areas so far surveyed, the gypsum accumulations coincide with a low yield of cacao, even though other physical and chemical characters of the soil appear to be favourable.

The results of manurial experiments suggest that a high gypsum content of the soil may prevent the proper utilisation of phosphate by the cacao tree. In view of the high percentage content of gypsum in ordinary superphosphate, this problem is of great fundamental importance where the cacao crop is concerned, since it suggests that the continued use of superphosphate over a long period of years might eventually give rise to harmful gypsum accumulations in the soil. Series II phosphate experiments [Section (A)] aim at testing this possibility, by comparing ordinary superphosphate, containing 50 to 60 per cent. of calcium sulphate, with other phosphatic manures, such as double superphosphate, which contain no calcium sulphate.

In addition, a special field experiment has been laid down to test the specific effect of heavy applications of calcium sulphate on the utilisation of phosphate and nitrogen, and on the uptake of iron by the cacao tree. The experiment consists of one 5 × 5 Latin Square laid out on a non-gypseous soil which is deficient in available phosphate. The different treatments are as follows:—(1) Control, (2) ammonium sulphate, (3) Nicifos C, (4) double superphosphate, (5) double superphosphate *plus* ammonium sulphate, with a half-plot calcium sulphate treatment.

Summary.

(1) Series I field manurial experiments were inaugurated in 1932 to test the specific effect of phosphatic manure on the growth and yield of cacao. The series consists of six separate experiments laid down on different soil types occurring in Trinidad. In each case the Latin Square type of lay-out was used.

(2) Records of yield for two complete crops since the application of the manures are presented for each experiment. All yield records have been subjected to statistical examination by Fisher's Analysis of Variance, and all positive conclusions and recommendations are based upon results which are statistically significant, with a minimum probability of twenty to one.

(3) The general results obtained have furnished conclusive proof that *the yield of mature cacao trees can be greatly increased on many different types of soil by the use of phosphatic manures alone.* Increased yields due to phosphate manuring have varied from 30 per cent. to over 100 per cent.

(4) The response of the cacao tree to phosphatic manure has varied considerably on the different soil types.

(5) On sandy soils, and on clay soils showing a faintly acidic or neutral reaction, the optimum application of superphosphate manure is 2 lb. per tree (600 lb. per acre).

(6) On markedly acidic clay soils, additional increases in yield may be obtained from an application of 3 lb. superphosphate per tree (900 lb. per acre).

(7) On gypseous clay soils, which show a high content of calcium sulphate in the sub-soil layers, the cacao trees have not benefited from the application of phosphatic manure, even though chemical analysis and pot tests have shown such soils to be deficient in available phosphate.

(8) Previous soil surveys have shown that a high calcium sulphate content of the sub-soil is a factor associated with poor growth and low yield of cacao in Trinidad.

(9) The results of the manurial experiments on gypseous soils suggest that the harmful effect of calcium sulphate in the soil may consist in preventing the proper utilisation of phosphate by the cacao tree.

(10) In view of the demonstrated need of the cacao tree for adequate supplies of phosphate, this possibility is of great fundamental importance in the proper manuring of the cacao crop. Ordinary superphosphate contains over 50 per cent. of calcium sulphate, and the findings suggest that the use of superphosphate in cacao cultivations over a long period of years might eventually give rise to harmful accumulations of gypsum in the soil.

(11) Further experiments have therefore been laid down to compare the effect of ordinary superphosphate with other types of phosphatic manures, such as double superphosphate, ground rock phosphate and ammonium phosphate, which contain no calcium sulphate. A special field experiment has also been laid down to test the effect of heavy dressings of calcium sulphate on the yield of cacao, and on the utilisation of phosphate and uptake of iron by the cacao tree.

(12) Further series of field manurial experiments are described, which have been recently laid down to test specific problems suggested by the results of previous investigations.

CACAO RESEARCH: CHEMICAL SECTION. LIST OF CACAO MANURIAL EXPERIMENTS IN TRINIDAD.

(Sites already set out in manured plots, or selected for the preliminary recording of natural yields) December, 1934.

Experiment.	Soil Type.	Age of Trees.	Lay-out.	Object.	First Application.
Series (A): Phosphate Trials.					
Las Hermanas I	Alluvial acidic clay	30-35 years	One 4×4 Latin Square	Superphosphate Interactions with Potash	May, 1932.
Las Hermanas II.	do.	do.	One 5×5 Latin Square	Comparison of Phosphatic Manures. Interactions with Potash & Nitrogen	May, 1934.
Las Hermanas III.	do.	do.	do.	Comparison of Phosphatic Manures. Interactions with Potash	April, 1935.
River Estate I.	Alluvial neutral sandy-silt	21 years	One 3×3 Latin Square	Superphosphate	May, 1932.
Rio Claro I.	Black calc. clay	25-30 years	do.	do.	Aug., 1932
Rio Claro II	Gypseous clay	do.	Two 3×3 Latin Squares	do.	do.
Rio Claro III.	Black calc. clay	20-25 years	Two 5×5 Latin Squares	Comparison of Phosphatic Manures. Interactions with Potash	April, 1935.
Series (B): General Manurial Trials.					
River Estate II	Alluvial neutral sandy-silt	20-25 years	One 5×5 Latin Square	Nitrogen, Phosphate, Potash :—Shaded Cultivation.	April, 1934.
River Estate III	do.	do.	do.	Nitrogen, Phosphate, Potash :— Unshaded Cultivation.	do.
River Estate IV	do.	do.	One 4×4 Latin Square	Mulch Interactions with Nitrogen, Phosphate and Potash	do.
River Estate V	do.	do.	One 5×5 Latin Square	Comparison of Ammonium Phosphate with Amm. Sulph. *plus* Superphosphate	April, 1935.
Rio Claro IV	Black calc. clay	do.	do.	Calc. Sulph. (gypsum). Interactions with Nitrogen and Phosphate	do.

PART III.

MULCHING EXPERIMENTS ON CACAO.

By J. A. McDonald.
(*Chemist for Cacao Research.*)

Introduction.

Soil surveys, carried out in the West Indies by the College Department of Chemistry, have shown that high yielding capacity of cacao soils is usually associated with a wide carbon/nitrogen ratio of the soil organic matter (1). Accordingly, the application of vegetable mulch to low-yielding soils as a means of widening the C/N ratio was recommended. The possibility that wide C/N ratios for high yielding soils may furnish an index, rather than a cause, of increased growth and yield has not been disregarded. Differences in the C/N ratio of good and bad cacao soils have been so marked and so consistent as to justify special experiments to test the effects of wide-ratio vegetable mulch on the yield of cacao, as well as on specific soil properties.

Many tropical crops have been shown to benefit from heavy applications of vegetable mulch. Experiments carried out in the West Indies (2), (3) have demonstrated that cacao and citrus respond to this treatment in Dominica, as also does arrowroot in St. Vincent. Benefit has also been obtained from mulching coffee in Uganda and rubber in Malaya. There is, however, little definite evidence as to the fundamental causes which underly these beneficial effects.

The effects of vegetable mulch on soil properties have recently been discussed in detail by Hardy (4), particularly in relation to the light-textured soils of St. Vincent. The effects may be classified into:—

(A) *Semi-permanent effects* on the physical and chemical properties of the soil, due to the addition of organic matter which takes place when the mulch decomposes. These effects may include (1) an increase in water-holding capacity of the soil, (2) an increased retentivity for exchangeable bases which are absorbed or fixed by colloidal humus, (3) the direct supply of nutrient elements, particularly nitrogen, by the decomposition of organic matter, and (4) the supply of growth-promoting substances, regarded by some authorities as essential to satisfactory plant growth. These semi-permanent results would probably not be effective until a considerable time had elapsed after the first application of the mulch. (B) *Temporary effects* on fluctuating soil factors. These effects may include, (1) possible conservation of soil moisture, (2) stabilisation of soil temperature, (3) increased activity of micro-organisms, and (4) increased rate of solution of mineral components of the soil, due to the liberation of carbon dioxide by the oxidation of organic matter.

The present series of mulch experiments on cacao are divided into two parts:—

Part I consists of two simple experiments, laid down at the end of 1932, with the definite purpose of providing fundamental information on the temporary effect of mulch on the moisture content, temperature and nitrogen status of typical cacao soils of Trinidad. The effect of mulch on the *yield* of cacao was not considered in these preliminary experiments. The natural variability in the yield of ordinary estate cacao is so great that yield records from a small number of plots may be valueless for comparing the effect of different treatments.

Part II consists of a more elaborate experiment, laid down in April, 1934, in the form of a Latin Square, on which it should be possible to obtain accurate statistical information on the effect of mulch on yield of cacao, as well as on various physical and chemical attributes of the soil.

The results obtained to date from the Part I experiments are presented in this article.

Part I: The Effect of Mulch on the Moisture Content, Temperature and Nitrogen Status of Typical Cacao Soils.

Review of Literature.

(*a*) *Effect of Mulch on Nitrification.*

Many investigations have been made in the past into the formation and accumulation of soil nitrates from nitrogenous organic matter. In most of these investigations, the organic matter has not been applied as a surface mulch, but has been incorporated into the soil.

When organic matter is added to soil, there may be a temporary depression in the supply of soil nitrate. The effects vary, however, depending on the carbon/nitrogen ratio of the organic matter. If the nitrogen content is relatively small (*i.e.*, non-leguminous, wide-ratio, fresh material) there may be a prolonged and severe depression of available soil nitrate. This is due to the development in the soil of a large population of cellulose-decomposing micro-organisms, which enter into competition with the plant, and assimilate a large proportion of the available soil nitrogen in the form of ammonia and nitrates. This assimilation may result in a temporary shortage of nitrogen to the growing plant, but, when sufficient carbohydrate has been decomposed to create a narrow C/N ratio, the rate of nitrification increases, so that the ultimate result of the addition of wide-ratio organic matter is an increased supply of soil nitrate.

If the initial nitrogen content of the added organic matter is relatively high (*i.e.* leguminous material or well rotted farm manure), there may be little or no depression in nitrate supply, which may show a marked increase shortly after the addition of the organic matter.

These characteristic effects on nitrate supply due to organic matter ploughed or forked into the soil are supported by a great deal of experimental evidence, and are now generally accepted. Comparatively few investigations have been reported, however, of the effect on nitrification of organic matter applied as a surface *mulch*. Scott (5) has shown that straw mulch applied to growing wheat at the rate of two tons per acre has a depressing effect on nitrate accumulation in the soil. Albrecht (6), (7) later showed that straw mulch applied to uncropped land caused a very marked depression of nitrates over a period of three years.

On the other hand, Beaumont and others (8), (9) have shown that the application of a surface mulch of waste hay and straw to the soil of a young apple orchard resulted in a pronounced accumulation of nitrate in the mulched soil. During the first three years of the experiment, there was little or no difference in the nitrate content of mulched and unmulched plots, but subsequently, there was a very marked accumulation of nitrate in the mulched soil. At no time was there any depression of nitrate in the mulched soil.

These opposing results may be readily explained by the different conditions under which the various experiments were conducted. In the experiments of Albrecht, the mulch was applied yearly, but, before each application, the partially-decomposed remains of the old mulch were raked off, and the land was ploughed. In Beaumont's experiments the mulch was also applied yearly, but the fresh mulch was applied on top of the old mulch, without disturbing the soil surface. In Albrecht's experiments, therefore, there was no chance of a layer of highly decomposed organic matter with narrow C/N ratio accumulating, such as would favour a high rate of nitrification.

The general conclusion from the above investigations is that organic matter applied as a surface mulch has much the same effect on the nitrate content of the soil as if the organic matter had been ploughed or forked in. As long as there is an abundance of fresh material having a wide C/N ratio in contact with the soil surface then a depression in the nitrate content of the soil takes place. When, however, a layer of highly decomposed material, having a narrow C/N ratio, has accumulated, there is a marked increase in the supply of soil nitrate. Beaumont's experiments show that, once the layer of highly decomposed material has accumulated on the soil surface, the increased nitrate supply is maintained, even though fresh mulch is subsequently applied. Beaumont suggests that nitrification takes place in the lower humified layer of mulch, rather than in the soil proper, and that the nitrates are carried into the soil by leaching.

(*b*) *Effect of Mulch on Soil Moisture.*

Much experimental work has been carried out, chiefly in the United States of America, on the effect of surface mulches in conserving soil moisture. The results of these experiments have recently been summarised and discussed by Keen (10). The majority of the investigations show that, if vegetative growth is restrained, the loss of water is practically the same from a mulched as from an unmulched surface. This is due to the fact that losses of water by evaporation from the soil surface are negligible compared with losses by transpiration from the growing plant. The effect of mulch in conserving moisture is therefore only incidental to the operation of destroying weeds.

Divergent results nevertheless have been obtained by certain other workers, who have shown that considerable losses of soil moisture can be directly prevented by maintaining a surface organic mulch. These divergent results are explained by Keen as being due to differences in the height of the permanent water-table. He has shown that upward capillary movement of water in soil is effective only over short distances. When the permanent water-table is six feet or more below the soil surface, soil moisture tends to distribute itself at a uniform moisture content throughout the soil, and upward movement of water is exceedingly slow. In such cases, mulching would have no direct effect in conserving soil moisture. When the permanent water-table is near the surface, however, mulch may be directly effective in conserving soil moisture.

The present series of mulching experiments on cacao differ from most of the above investigations in that they were carried out on a soil carrying an actively growing crop. No special control of weeds on the unmulched plots was attempted, the weeds on both plots being cutlassed about once every four months, according to ordinary estate practice. Under the conditions existing in the cacao fields, the mulch was effective in greatly reducing the growth of weeds, and from this consideration alone one would expect the mulched plot to show a generally higher moisture content than the controls.

(*c*) *Effect of Mulch on Soil Temperature.*

A layer of litter or mulch on the soil surface reduces the daily fluctuation in soil temperature (11). This effect is most pronounced actually at the soil surface, and decreases at lower depths.

Mackinney (12) has shown that a two-inch layer of forest litter (pine needles) caused a general increase in soil temperature down to a depth of 36 inches and also greatly reduced the fluctuations in maximum and minimum surface soil temperatures, the diurnal range being reduced from 60 to 80 per cent. The litter cover did not influence daily variation in soil temperature below six inches depth.

Under tropical conditions, the surface soil may be heated to very high temperatures by the sun, and the effect of mulch or surface litter in stabilising surface soil temperatures may be of great importance. Leather (13) states that the maximum temperature at Pusa, India, may rise to 158°F at the soil surface, and 122°F at a depth of two inches. McKenzie Taylor and Burns (14) have recorded 149°F at the surface, and 133°F at two-inch depth at Giza, near Cairo. Temperature fluctuations below three inches depth are probably of less importance in the Tropics. In Trinidad, soil temperatures at three inches depth in an open pasture show a diurnal and annual range of only 10°F, the maximum temperature seldom exceeds 90°F while the minimum seldom falls below 70°F. Direct sunshine is the chief cause of the big variations in surface soil temperatures in the Tropics, and, in situations where the ground is well shaded, fluctuations in surface soil temperature will normally be much less than in the open. In cacao fields in Trinidad, the ground is shaded, both by the cacao trees themselves, and by the special shade trees. There is also a layer of natural litter on the soil surface. The data in Table III show that the mean soil temperature at three inches depth in cacao fields is 5°F less than in the open, the mean diurnal range of soil temperature being reduced from 7.2°F in the open to 2°F under cacao. The application of vegetable mulch, therefore, could have only a small differential effect on surface soil temperatures under these conditions

(*d*) *Paper Mulches.*

The Hawaiian practice of applying a paper mulch to sugar-cane and pine-apple fields has not been included in this discussion, since the practice has many fundamental differences from the application of ordinary vegetable mulches. The paper used is impregnated with tar or asphalt, so that it forms a soil cover which is quiet impervious to light, air and moisture. In addition, no organic matter is added to the surface soil as is the case when ordinary vegetable mulches decompose. The greatest practical value of paper mulches appears to be due to the control of weeds, but many Hawaiian workers have shown, that apart from weed control, paper mulch has definitely beneficial effects on the growth of sugar-cane and pine-apples. Stewart (15) and others have shown that application of paper mulch results in an increase in soil moisture, soil temperature, and the supply of ammonia and nitrate nitrogen.

Experimental Conditions.

(*a*) *Soil Type.*

For the present series of experiments, plots were selected on two contrasted soil types. The chief physical and chemical characteristics of the first six-inch layer of soil are shown in Table I, while the texture of the soil at different depths is shown in Table II.

Table I.

Sites of Mulch Experiments; Composition of Top Six-inch Layer of Soil.

Soil Type and Locality.	Sand.	Index of Texture.	Reaction		O.M.	N.	C/N Ratio.	Rate of Soln.	Avail. K_2O	Avail. P_2O_5
			Norm.	Exch.						
	%	(I.T.)	pH.	pH.	%	%			p.p.m.	
Torrecilla. (1) Sandy Alluvium, Northern Range, Trinidad	53.7	23	6.7	6.2	1.80	0.16	6.5	—	150	21
Las Hermanas. (2) Clay Alluvium, Caroni Plain, Trinidad	9.4	47	6.2	5.4	2.99	0.27	6.0	201	139	29

Table II.

Sites of Mulch Experiments; Soil Texture down to 4-ft. Depth.

Lower Depth Inches.	Torrecilla.		Las Hermanas.	
	% Sand.	Index of Texture*	% Sand.	Index of Texture.
1½	53.2	25	11.5	49
3	53.4	23	9.0	47
6	54.2	22	8.2	46
9	52.0	22	8.3	43
12	51.0	20	5.7	43
15	50.5	20	6.0	42
18	47.2	19	6.0	42
24	46.4	19	4.0	42
30	51.0	19	3.5	38
38	50.5	18	3.0	37
50	49.8	18	5.5	36

* Index of Texture = Sticky Point Moisture − 1/5 Sand Content.

The soil type at Las Hermanas is a fairly heavy-textured clay, while at Torrecilla, the soil is a light sandy-loam, which is mixed with coarse gravel below a depth of 2 feet.

(b) Environmental Conditions.

A correct appreciation of the environmental conditions which obtain in normal cacao fields in Trinidad is essential for the accurate interpretation of the results of these experiments. The ground is covered by a surface layer of litter, which consists of dead leaves fallen from cacao trees and from leguminous Immortel shade trees, mixed with the remains of various types of shade-loving plants which grow as weeds among the cacao trees, and which are cut and left on the ground as a routine estate practice. The ground is heavily shaded, both by the canopy of the cacao trees themselves, and by the special shade trees. Climatic and environmental conditions are shown by the data in Table III.

Table III.

Summarised Meteorological Data from Sites of Mulching Experiments, and, for Comparison, from College Meteorological Station.

Site.	Season.	Rainfall Total inches.	Relative Humidity Average Minimum. %	Rate of Evaporation c.c. per 24 hrs.	Radiation cc. per 24 hrs.	Air Temperatures.			Soil Temperatures.		
						Average Maximum.	Average Minimum.	Average Range.	Average Maximum.	Average Minimum.	Average Range.
Torrecilla	Jan.-June	19.15	56.8	13.3	2.5	84	72	12	78.6	75.9	2.7
Cacao Field	July-Dec.	67.23	67.7	7.4	1.5	81	73	8	77.2	75.0	2.2
Las Hermanas	Jan.-June	17.24	50.2	13.4	5.2	85	69	16	77.8	75.8	2.0
Cacao Field	July-Dec.	74.61	69.2	5.6	3.3	83	70	13	76.6	75.6	1.0
Imperial College	Jan.-June	16.28	51.2	38.5	18.0	85	69	16	85.0	77.6	7.4
Open Pasture	July-Dec.	44.35	60.9	21.1	14.7	87	72	15	85.0	78.0	7.0

The data in Table III show an environment of high rainfall, very high humidity, high temperature, low evaporation and low light intensity. Conditions at the soil surface in Trinidad cacao fields are therefore closely akin to tropical rain forest, and differ markedly from the conditions obtaining in temperate fruit orchards and in other tropical orchard crops, such as citrus and rubber.

(c) Plot Lay-out and Treatments.

At each site, four plots were selected, each plot being $\frac{1}{6}$-acre in area, and containing approximately 50 cacao trees. Two plots at each site were kept as controls, and two plots were mulched at the rate of 80 tons fresh mulch per acre. At Las Hermanas, the first application of mulch was made in December, 1932, a second application being made in December, 1933. At Torrecilla, the first application was made in April, 1933, and the second application in January, 1934.

(d) Composition of Mulch.

The mulch consisted of mixed vegetation, chiefly various types of grasses, such as can easily be obtained from the edges of forest land, or from areas carrying secondary scrub near the cacao fields. The composition of the fresh mulch and the partially decomposed mulch is shown in Table IV.

Table IV.

Sites of Mulch Experiments: Composition of Fresh Mulch and Partially Decomposed Mulch.

Experimental Site.	Material.	%Moisture in Fresh Material			%Carbon in Dry Matter			%Nitrogen in Dry Matter			Carbon/ Nitrogen Ratio.		
		A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Torrecilla	Fresh Mulch	64.4	65.1	64.8	47.6	46.9	47.3	0.495	0.536	0.515	96.2	87.6	91.4
	Decomposed Mulch : Upper Layer	—	—	—	44.9	40.0	42.4	0.522	0.605	0.563	86.2	66.2	76.2
	Decomposed Mulch : Lower Layer	—	—	—	32.4	34.5	33.4	2.19	2.16	2.17	14.8	16.0	15.4
Las Hermanas....	Fresh Mulch	71.6	73.7	72.7	45.5	46.0	45.7	0.605	0.618	0.611	75.3	74.5	74.9
	Decomposed Mulch : Upper Layer	—	—	—	39.2	28.7	33.9	0.887	0.838	0.862	44.2	34.2	39.2
	Decomposed Mulch : Lower Layer	—	—	—	30.4	27.5	28.9	1.99	1.96	1.98	15.2	14.0	14.6

Note.—Fresh mulch applied December, 1932, and December, 1933. Samples of partially-decomposed mulch taken November, 1934. Percent Carbon was determined by the method of Robinson, McLean and Williams, results being corrected by factor 1.116.

The data in Table IV show that the fresh mulch has a relatively very low nitrogen content and a wide carbon/nitrogen ratio. There is a difference in the composition of the mulching material from the two sites. The Torrecilla mulch has a larger proportion of woody material (lower moisture content), a lower nitrogen content, and a wider C/N ratio than the Las Hermanas mulch.

(*e*) *Method of Sampling for Moisture and Nitrate Determinations.*

The first soil samples were taken at each site one month after the first application of mulch, and thereafter every two weeks until the end of June, 1934. The samples were procured in each plot at three different depths, as follows:—(1) first six-inch layer, (2) second six-inch layer, and (3) second foot layer, each sample being composited from ten different spots within each plot. Moisture determinations were carried out on all the routine samples. In addition, an attempt was made to study the moisture profile down to a depth of eight feet by means of special samples taken at definite intervals throughout the year. At Las Hermanas, this was found to be a simple procedure, since the soil is of fairly homogeneous texture down to the eight foot level. At Torrecilla, however, the presence of a large proportion of stones and gravel below the two-foot level makes soil sampling for moisture determination extremely difficult; at this site, therefore, only one set of special depth samples was taken in order to obtain a rough idea of the distribution of sub-soil moisture in the mulched and unmulched plots.

Nitrate determinations were made every four weeks, but only on the samples from the first six-inch layer of soil, previous investigations having shown that the activity of nitrifying organisms of cacao soils is mainly confined to the top six-inch layer. In addition to determining the nitrate content of the fresh soil, the nitrifying ability of the soil was measured by two different methods:—(*a*) A portion of the soil sample was stored at field moisture content for a period of four weeks, and nitrate was then determined, (*b*) a fixed quantity of ammonium sulphate was added to the soil, which was then stored at field moisture content, and nitrate determined. In both cases, the moisture content of the soil was kept constant during storage by periodic weighing, any moisture lost by evaporation being replaced. Nitrates were determined by the phenol disulphonic acid method.

Ammonia determinations were carried out on a number of the periodic samples, but no more than traces of ammonia were ever found. In this connection, Russell (16) states that the ammonia formed by the action of soil bacteria or added in manures is rapidly converted into nitrite and nitrate, the changes proceeding so rapidly that, in many soils, only traces of ammonia or nitrite are ever found. The production of nitrite is slower than that of nitrate while the formation of ammonia is slower still, and sets a limit to the rate at which the process can take place. Thus, a measure of the rate at which nitrates are formed in soil does not measure the rate of nitrification, as is sometimes assumed,

but the rate of ammonia production. Method (*a*) therefore gives a measure of the rate of ammonia production, or the ability of the soil micro-organisms to produce ammonia and nitrate from the soil organic matter. Method (*b*), on the other hand gives a measure of their ability to produce nitrate, when abundantly supplied with ammonia. Both methods give a measure of the rate of production of nitrogenous compounds which are readily available to the plant. Previous investigations have shown that, even in a soil in which nitrates are being abundantly produced, the nitrate content of fresh samples may be consistently very low, due (*a*) to rapid removal of nitrate by the roots of growing plants, and (*b*) to rapid leaching. In an investigation of this nature, therefore, methods which measure the rate of production of ammonia and nitrate in stored samples are more likely to yield useful information than measurement of the nitrate content of fresh soil.

(*f*) *Soil Temperature Measurements.*

Soil temperature measurements were carried out by means of recording thermometers of the mercury-in-steel type. The instruments used were Model M. 2236, supplied by Messrs. Negretti and Zambra, Ltd., London. The bulb of the thermometer was placed horizontally at a depth of three inches below the soil surface. Because of their expense, only two of these instruments were obtained, and soil temperature measurements were carried out on two of the plots at Las Hermanas Estate only.

Results.

I.—Effect of Mulch on Production of Available Nitrogen.

(*a*) *Experimental Data.*

From considerations of space, the actual data on the nitrate content of the soil from mulched and unmulched plots are not presented in this article, but the results of their statistical examination are summarised in Table V.

Table V.

Mean Nitrate Content of Top Six-inch Layer of Soil from Mulched and Unmulched Plots.

	Mean Nitrate Content ; p.p.m.					
	Fresh Soil.		Soil Stored 4 weeks at Field Moisture Content.		Soil + $(NH_4)_2SO_4$ Stored 4 weeks at Field Moisture Content	
	Las Hermanas.	Torrecilla.	Las Hermanas.	Torrecilla.	Las Hermanas.	Torrecilla.
Mulch A	9.1	13.7	42.9	41.3	209	248
Mulch B	8.7	13.0	44.7	43.7	200	249
Mean A+B	8.9	13.3	43.8	42.5	204	249
Control A	7.0	10.6	34.6	29.0	145	150
Control B	6.6	9.6	32.8	28.0	133	145
Mean A+B	6.8	10.1	33.7	28.5	139	148
Difference Between Means	2.1	3.2	10.1	14.0	65	101
Standard Error of Difference Between Means	0.9	1.3	3.0	2.8	12.3	16.7

Each value for the Las Hermanas Site is the mean of 18 determinations on soil samples taken every four weeks over a period of 18 months from January, 1933 to June, 1934. Each value for the Torrecilla Site is the mean of 15 determinations on soil samples taken every four weeks over a period of 14 months from May, 1933 to June 1934. A difference between two means equal to 2.04 times the Standard Error may be considered significant, with a minimum probability of 20 : 1.

(*b*) *Discussion of Results.*

At both sites, the application of a wide-ratio vegetable mulch has resulted in an immediate very large increase in the production of soil nitrate, the increase being very marked after a lapse of only six weeks, and being maintained immediately after a second application of fresh mulch. The increased nitrate supply in the mulched plots is clearly shown by the nitrate content of the fresh samples, but even more marked differences are shown in the rate of ammonia production, and the rate of nitrification. It

would appear that the application of mulch has resulted in a greatly increased activity of soil micro-organisms responsible for the production of ammonia and nitrate. The rate of nitrification greatly exceeds the rate of ammonia production, so that ammonium compounds do not accumulate in the soil, only traces of ammonia being found in soil samples.

Review of the literature of the subject indicates that these results are not exceptional. Beaumont and others have shown that, once a layer of highly decomposed organic matter has been formed, there is a marked increase in the rate of nitrification and in nitrate accumulation in mulched soil. This increased nitrification is maintained when applications of fresh mulch are subsequently made. In cacao fields, there is normally a layer of highly-decomposed organic matter on the soil surface, which accounts for there being no temporary depression in nitrate supply. The applied mulch has the effect merely of maintaining and increasing the already existing layer of highly decomposed organic material.

The very marked increase in the supply of available soil nitrogen in the mulched plots may be indirectly caused by the effect of the mulch on other soil factors. Quite apart from the organic matter factor, the soil cover provided by a vegetable mulch seems to produce conditions in the surface layers of soil which are particularly favourable to the activity of ammonia-producing and nitrifying organisms. The particular factors involved are probably soil moisture and soil temperature. In the following sections, it will be shown that, for the present series of experiments, the conservation of soil moisture by the mulch is probably of greater importance in increasing nitrate production, than the stabilisation of soil temperature.

II.—Effect of Mulch on Soil Moisture.

(*a*) *Experimental Data.*

From considerations of space, the actual data of the moisture content of the soil from mulched and unmulched plots are not here recorded, but the results of their statistical analysis are set out in Table VI.

Table VI.

Mean Moisture Content of the Soil from Mulched and Unmulched Plots.

	Available Soil Moisture.					
	1st 6-inch Layer.		2nd 6-inch Layer.		2nd Foot Layer.	
	Las Hermanas.	Torrecilla.	Las Hermanas.	Torrecilla.	Las Hermanas.	Torrecilla.
Mulch A	23.4	24.8	15.6	14.0	14.2	11.2
B	24.6	24.9	15.3	14.0	13.8	11.0
Mean	24.0	24.8	15.4	14.0	14.0	11.1
Control A	21.5	12.2	14.4	7.2	13.8	8.3
B	21.9	11.8	14.3	7.4	13.9	8.4
Mean	21.7	12.0	14.4	7.3	13.8	8.4
Difference Between Means	2.3	12.8	1.0	6.7	0.2	2.7
Standard Error of Differences Between Means	0.8	0.7	0.6	0.6	0.4	0.4

Each value for the Las Hermanas Site is the mean of 36 determinations on soil samples taken every two weeks over a period of 18 months from January, 1933 to June, 1934. Each value for the Torrecilla Site is the mean of 27 determinations on soil samples taken every two weeks over a period of 14 months from May, 1933 to June, 1934. A difference between two means equal to twice the Standard Error may be considered significant, with a minimum probability of 20 : 1.

(*b*) *Discussion of Results.*

At Torrecilla Estate (sandy soil) the average soil moisture content of the mulched plots is significantly larger than that of the unmulched plots. The increased moisture content of the soil of the mulched plots is pronounced at all depths down to two feet, but the increase is much larger in the first six-inch layer than in the second six-inch, or in the second foot of soil.

At Las Hermanas Estate (clay soil), the moisture content of the first six-inch layer of soil in the mulched plots is significantly higher than that of the unmulched plots, but the difference is very small. There is no significant difference in the moisture content of the second six-inch layer and second foot of soil from mulched and unmulched plots at Las Hermanas.

Table VII.

Sites of Mulch Experiments. Moisture Profile Data. Soil Moisture at Different Levels down to 8 ft., at Different Seasons (Las Hermanas). During Wet Season only (Torrecilla).

Lower Depth inches	Gross Soil Moisture : Per cent.													
	Las Hermanas.												Torrecilla.	
	Mulch						Control.						Mulch.	Control.
	15-2-33	25-4-33	23-11-33	28-3-34	20-6-34	Mean	15-2-33	25-4-33	23-11-33	28-3-34	20-6-34	Mean		
2	60	42	72	55	42	54	56	39	65	52	30	48	56	44
4	—	—	51	48	41	47	—	—	54	47	31	44	46	38
6	—	—	47	44	40	44	—	—	48	43	39	43	40	34
8	42	35	46	45	40	41	41	33	47	41	35	40	34	33
10	—	—	45	45	38	42	—	—	44	41	35	40	30	28
12	—	—	42	38	37	39	—	—	42	39	36	39	31	27
14	—	—	42	38	36	39	—	—	42	39	34	38	26	25
16	36	32	39	37	39	37	38	32	42	37	34	37	25	26
24	32	28	36	36	31	33	35	31	37	33	38	35	23	27
32	32	28	32	39	35	33	34	33	36	34	33	34	23	26
40	34	29	32	36	34	33	34	31	37	34	33	34	23	25
48	33	32	35	36	31	33	32	31	35	34	32	33	22	24
56	—	—	34	36	33	34	—	—	34	33	35	34	24	25
64	—	—	33	30	34	32	—	—	34	35	35	34	23	21
72	—	—	31	31	35	32	—	—	33	31	33	32	23	21
80	—	—	32	29	35	32	—	—	32	31	33	32	—	—
86	—	—	33	31	33	32	—	—	32	33	34	33	—	—

The moisture profile data in Table VII show that the increased soil moisture of the mulched plots at Las Hermanas may be confined to an even shallower layer than the first six inches. The increase in moisture is most marked in the surface two inches, but, below four inches depth, there is no marked difference in the soil moisture content of mulched and unmulched plots. The moisture profile data also show that, in the heavy clay soil at Las Hermanas, seasonal fluctuations in soil moisture are mainly confined to the surface two foot layer; below a depth of two feet, soil moisture fluctuates very little throughout the year, the downward percolation of water being evidently very slow. The permanent water-table at both plots evidently lies well below the six-foot level.

The reasons for the great difference in results between the two sites is not quite clear. At neither site is the permanent water-table within six feet of the surface, and according to the arguments put forward by Keen, mulch should have little effect in directly preventing water loss from the soil surface. The small differences in moisture content noted at Las Hermanas may be due solely to the increased growth of weeds on the unmulched plots, but the very large differences obtained on the sandy soil at Torrecilla suggest that here the mulch has had a direct effect in conserving soil moisture.

III.—Relationship between soil moisture and Nitrate Production.

(*a*) *Experimental Data.*

The results presented in previous sections show that mulching two typical cacao soils has resulted in a greatly increased rate of nitrate production, as well as an increased moisture content of the surface soil. This suggests that the nitrate content of these soils, and the activity of the ammonia-producing and nitrifying organisms may be directly correlated with soil moisture. The correlation between soil moisture and nitrate content of all the experimental plots has been calculated, and the results are presented in Table VIII.

(*b*) *Discussion of Results.*

(1) On the *sandy soil* at Torrecilla, there is a significant negative correlation between moisture content and the nitrate content of the *fresh* soil, both in mulched and unmulched plots, *i.e.*, increased moisture content is accompanied by decreased nitrate accumulation in the soil. This suggests that, in sandy soil, nitrates are very rapidly leached away during wet weather, and, even though the rate of nitrification may increase, the nitrate content of the fresh soil may actually be very low in consequence. Measurement of the nitrate content of fresh samples alone will therefore give a false indication of nitrate supply in this type of soil.

Table VIII.

Relationship between Soil Moisture and Production of Soil Nitrate in Mulched and Unmulched Plots.

Experimental Site.	Plot Treatment.	Factors Correlated.	Correlation Coefficient (r).	Number Pairs of Determinations (n).
Torrecilla	Mulch	Soil Moisture and NO_3 Content of Fresh Soil	—0.63	30
		Soil Moisture and NO_3 in Soil Stored 4 weeks	+0.13	30
		Soil Moisture and NO_3 in Soil + $(NH_4)_2SO_4$ Stored 4 weeks	+0.69	30
	Control	Soil Moisture and NO_3 Content of Fresh Soil	—0.48	28
		Soil Moisture and NO_3 in Soil Stored 4 weeks	+0.28	28
		Soil Moisture and NO_3 in Soil + $(NH_4)_2SO_4$ Stored 4 weeks	+0.71	28
Las Hermanas	Mulch	Soil Moisture and NO_3 Content of Fresh Soil	+0.08	36
		Soil Moisture and NO_3 in Soil Stored 4 weeks	+0.28	36
		Soil Moisture and NO_3 in Soil + $(NH_4)_2SO_4$ Stored 4 weeks	+0.67	36
	Control	Soil Moisture and NO_3 Content of Fresh soil	+0.42	34
		Soil Moisture and NO_3 in Soil Stored 4 weeks	—0.17	34
		Soil Moisture and NO_3 in Soil + $(NH_4)_2SO_4$ Stored 4 weeks	+0.42	34

If the value of r exceeds 0.38, a significant correlation between the two factors is indicated, with a minimum probability of 20 : 1.

On the other hand, for the sandy soil, there is a significant positive correlation between the *rate* of nitrification and soil moisture content, *i.e.*, increased soil moisture is accompanied by increased activity of the nitrifying organisms, and an increased rate of nitrate production. There is no definite relationship between moisture content of the soil and rate of ammonia production.

(2) On the *clay soil* at Las Hermanas, there is a significant positive correlation between moisture and nitrate content of the soil of the control plots. On the mulched plots, however, there is no definite relationship between these two factors. No satisfactory explanation of this result can be put forward at present, but it is clear that leaching of nitrate during wet weather is not so rapid in the heavy clay soil at this site. There is a significant positive correlation between moisture content and rate of nitrification on both mulched and unmulched plots, *i.e.*, increased soil moisture is accompanied by increased rate of nitrification. There is no definite relationship between soil moisture and rate of ammonia production.

The general conclusions to be drawn from these results are that an increased moisture content of the surface soil at both sites favours an increase in the rate of nitrification. Nitrification takes place mainly in the surface six-inch layer of soil, and the greatest activity of nitrifying organisms is probably confined to an even shallower layer. Since the application of mulch has had the effect of increasing the moisture content of the surface layers of soil, the increased nitrate supply in the mulched plots may be due partly to their increased and more stable moisture content.

IV.—Effect of Mulch on Soil Temperature.

(*a*) *Experimental Data.*

Continuous records of soil temperature at a depth of three inches in mulched and unmulched plots are available for a period of 22 months. Records were taken at the Las Hermanas site only. The actual data are not tabulated, but the monthly records were examined by statistical methods ; the results are presented in Table IX.

(*b*) *Discussion of Results.*

Daily and seasonal fluctuations in the temperature of the surface soil in normal cacao fields in Trinidad are very small. There are slight differences only in maximum, minimum and daily range of soil temperature in mulched and unmulched plots. The mulched plots have a lower maximum, a higher minimum, and a smaller daily range of soil temperature than the unmulched plots, but the differences are so small that it would not be reasonable to attribute any

Table IX.

Las Hermanas Estate: Mean Soil Temperature at Three Inches Depth in Mulched and Unmulched Plots.

	Soil Temperature: Degrees Fahrenheit.		
	Max. Temp.	Min. Temp.	Range. Max.-Min.
Mulch	76.7	75.8	0.9
Control	77.1	75.6	1.5
Difference	0.4	0.2	0.6
Standard Error of Difference	0.3	0.3	0.2

Each temperature value is the general mean obtained from continuous records taken over a period of 22 months, from January, 1933 to October, 1934. A difference between two means equal to 2.07 times the Standard Error may be taken as significant, with a probability of 20 : 1.

possible effects on growth or yield of cacao, or any differences in micro-organic activity, to the effect of mulch in stabilising soil temperature.

The data, however, do indicate the general sort of effect that mulch may exert on soil temperatures at a depth of three inches. Under more exposed conditions, *i.e.*, in arrowroot plantations, or in citrus orchards, mulch may be expected to have a similar but more marked stabilizing effect on temperature fluctuations in the surface layers of soil, and these effects may be sufficiently pronounced to influence the activity of soil micro-organisms on the growth of crop plants. In addition to reducing fluctuations in surface soil temperatures, mulch appears to cause a general *decrease* in the mean temperature of the surface soil under tropical conditions. This is the opposite of the effect observed in cold climates, where mulch causes a general *increase* in the mean temperature of the surface layers of soil (11), (12).

General Conclusions.

The most striking effect of mulching two typical cacao soils in Trinidad has been an immediate large increase in the supply of soil nitrate. This increased nitrate supply was very marked and very consistent on two contrasted types of soil, and, in view of the results obtained by other investigators, it appears that the application of a vegetable mulch will generally result in an increased supply of soil nitrate. There may be a temporary depression in nitrate supply before the increase becomes permanent, (depending partly on the environmental conditions, and partly on the carbon/nitrogen ratio of the added material), but, once a layer of partially decomposed organic matter has become established on the soil surface, the increased nitrate supply will be maintained when subsequent applications of fresh mulch are made. In this connection, manurial experiments in St. Vincent have shown that large increases in the yield of arrowroot may be obtained, either by mulching, or by supplying artificial nitrogenous manures, suggesting that the beneficial effect of mulch in this instance lies in an increased supply of available nitrogen. In cacao fields in Trinidad, where there is an already existing layer of highly-decomposed organic matter on the soil surface, there is no temporary depression in nitrate supply, the increased nitrate in mulched plots being very marked after a lapse of only six weeks. The increased nitrate production in mulched plots seems to be due partly to the provision and maintenance of a layer of highly-decomposed organic matter on the soil surface in which nitrifying organisms are particularly active, and partly to indirect effects on moisture and temperature, due to the soil-cover provided by the mulch.

In the present series of experiments, the mulching has resulted in a marked conservation of moisture in the surface layers of soil, the increase in moisture being much more pronounced, and extending to a much greater depth, in a sandy soil than in a clay soil. There is also evidence that an increased moisture content of the soil coincides with an increased rate of nitrate production, suggesting that the increased nitrate content of the mulched plots may be partially due to increased and more stable moisture conditions in the surface layers of soil where nitrifying organisms are most active.

Mulching has also resulted in lessening the fluctuations in surface soil temperatures. Under the conditions of these experiments, the differential effects are very small, but under more exposed conditions, the effect of mulch in stabilising soil temperatures may be sufficiently pronounced to influence micro-organic activity and the growth of crop plants.

These preliminary experiments have provided exact information on the effects of mulch on moisture, temperature and nitrogen supply of the soil. The effects may be modified or exaggerated depending upon environmental conditions, but it seems likely that, as a general principle, the results will be fundamentally similar, even though soil, climatic and environmental factors may differ. No data are yet available regarding the effect of mulch on yield of cacao in Trinidad, but the mulching experiments are being continued and extended, in order that the effect of mulch on yield may be studied, as well as its effect on the permanent fertility of the soil.

Summary.

(1) Mulching experiments on cacao are described, in which the effects of mulch on the moisture content, temperature, and nitrogen status of the soil have been studied.

(2) Under certain conditions, the application of a wide C/N ratio vegetable mulch may be followed by a temporary depression in the supply

of available soil nitrogen, but, once a layer of partially decomposed organic matter has accumulated on the soil surface, an increase in the rate of nitrate production occurs. This increase is maintained when applications of fresh mulch are subsequently made.

(3) In cacao fields in Trinidad, no temporary depression in soil nitrate content follows the application of organic mulches, since a layer of highly decomposed organic matter already exists on the soil surface.

(4) The application of mulch to two typical cacao soils has resulted in the following effects :—

(*a*) An immediate large increase in the supply of soil nitrate.

(*b*) An increased moisture content of the surface layers of soil, the increase being much more marked, and extending to a much greater depth in a sandy soil than in a clay soil.

(*c*) A smaller daily fluctuation in surface soil temperatures.

(5) In all the experimental plots, both mulched and unmulched, an increased soil moisture content coincides with an increased production of soil nitrate, the quantitative relationship being statistically significant.

(6) The increased supply of soil nitrate in mulched plots appears to be due partly to the provision and maintenance of a layer of highly decomposed organic matter on the soil surface, and partly to the increased and more stable moisture content of the surface layers of soil, which also favours an increased activity of nitrifying organisms.

(7) The probable effects of mulch on nitrate production, moisture content and temperature fluctuations in the soil, under different conditions of soil and environment, have been discussed. It is concluded that the effects may be modified or exaggerated, depending on environmental conditions, but will generally be fundamentally similar to the results obtained in these experiments.

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PART IV.

THE EFFECT OF MANURIAL TREATMENTS ON THE CHEMICAL COMPOSITION OF CACAO LEAVES: THE DIAGNOSIS OF SOIL AND CROP NUTRIENT REQUIREMENTS BY MEANS OF LEAF ANALYSIS.

By J. A. McDonald and G. Rodriguez (*Analyst*).

Introduction.

The possibility of diagnosing soil nutrient deficiencies and the nutrient requirements of the cacao tree by means of chemical analysis of leaves was fully discussed in the Third Annual Report on Cacao Research. Preliminary results were then published of chemical analyses of cacao leaves taken from experiment plots receiving different phosphate and potash treatments. No experimental plots receiving nitrogen treatments were then available, so that it was not possible to study the effect on leaf composition of nitrogen applications. In the preliminary investigations, the leaf samples were few in number, being composite samples taken from all plots receiving identical manurial treatments. It was not possible, therefore, to interpret the results by means of statistical methods, or to arrive at really definite conclusions concerning the possibilities of this method for diagnosing the nutrient requirements of the cacao tree. In spite of the somewhat limited scope of the investigations, the experimental results were nevertheless sufficiently suggestive to warrant more detailed investigation.

With the co-operation of the Botanical Section of Cacao Research, leaf samples have been taken from a complex field manurial experiment, involving the use of nitrogen, phosphate and potash treatments, both alone and in combination. The different manurial treatments have now been applied for two successive years, so that the material available is eminently suitable for obtaining definite evidence regarding the full effects of manurial treatments on the composition of cacao leaves. This experiment was designed and laid out by the Botanical Section in co-operation with the Trinidad Department of Agriculture. The design, the scheme of treatments and one year's yield results are presented and discussed in detail by F. J. Pound in an article in the Botanical Section of this report. Chemical analysis of the soil and the experimental results obtained by Pound show that the soil at this site is characterised by a marked deficiency in *potash*. On the other hand, the soil of the phosphate experiment, from which previous leaf samples were obtained, is markedly deficient in *phosphate*. Data for leaf analyses obtained from the present experiment should therefore provide an interesting comparison with the data previously reported. Since separate leaf samples were taken in this investigation, it has been possible to correlate leaf composition with yield for each individual plot, and thus to obtain an accurate index of that particular leaf composition which corresponds with optimum nutrient supply as shown by maximum yield.

Experimental Procedure and Methods of Sampling.

Full details concerning the layout of the experiment are given by Pound (Botanical Section p. 19). The experiment consists of one 4×4 Latin Square with sixteen main plots, each containing sixteen trees in a 2×8 arrangement. Each main plot is subdivided so as to receive a half-plot treatment. Considering half-plots, there are 32 individual plots each containing eight trees in a 2×4 arrangement. Guard rows are provided between the main plots, but not between each pair of half-plots. In taking leaf samples, the four trees bordering the division in the middle of the main plot were ignored, and samples were taken only from the six end trees in each half-plot. Duplicate samples of young leaves, which had just hardened and turned green, were taken from each half-plot, making a total of 64 separate samples for chemical analysis.

The individual leaf samples were examined in the laboratory by the analytical staff of the College Chemistry Department for total ash, nitrogen, phosphate and potash content. In the following sections, the results of these determinations are presented and discussed. In is intended later to determine the calcium, magnesium, iron, sulphate and chloride contents of all the samples, but these analyses will not be completed in time for inclusion in this report.

Results.

I.—Accuracy of Sampling.

(*a*) *Experimental Data.*

The collected analytical results are presented in Table I. Fig. I shows a plan of the experiment, and provides a key to the different manurial treatments, and to the plots from which the different leaf samples were taken. Table II shows the variation in chemical composition of leaf samples from different plots, compared with the variation between duplicate samples. The particular index of leaf composition selected for this comparison is the nitrogen/potash ratio, since previous investigations have shown that this ratio may be the most accurate index of nutrient supply.

Table I.

Chemical Composition of Cacao Leaves: Analytical Data.

Plot No.	% Ash in Dry Matter.			Composition of Ash.									Nutrient Ratios.								
				% N			% P_2O_5			% K_2O			N/P_2O_5			N/K_2O			K_2O/P_2O_5		
	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
1	6.50	6.20	6.35	33.0	34.2	33.6	9.6	10.1	9.8	32.3	31.8	32.0	3.4	3.4	3.4	1.02	1.08	1.05	3.4	3.1	3.3
2	6.48	6.79	6.63	35.7	35.2	35.4	10.4	9.7	10.1	28.0	25.9	27.0	3.4	3.6	3.5	1.27	1.36	1.32	2.7	2.7	2.7
3	5.69	5.75	5.72	39.9	39.8	39.9	11.4	11.3	11.3	29.5	29.5	29.5	3.5	3.5	3.5	1.35	1.34	1.34	2.6	2.6	2.6
4	6.85	6.71	6.78	34.6	35.0	34.8	10.9	10.4	10.7	32.1	31.6	31.9	3.2	3.4	3.3	1.07	1.11	1.09	2.9	3.1	3.0
5	8.59	8.56	8.58	26.8	25.9	26.4	6.9	6.9	6.9	34.7	35.5	35.1	3.9	3.8	3.8	0.77	0.73	0.75	5.0	5.2	5.1
6	7.49	7.72	7.60	29.4	28.6	29.0	7.8	7.9	7.8	33.0	34.2	33.6	3.8	3.6	3.7	0.89	0.84	0.86	4.2	4.3	4.2
7	7.80	7.88	7.84	31.8	30.0	30.9	8.2	8.1	8.1	35.1	34.3	34.7	3.9	3.7	3.8	0.91	0.87	0.89	4.3	4.3	4.3
8	7.70	7.59	7.64	27.5	28.0	27.7	8.0	8.2	8.1	36.1	37.6	36.8	3.4	3.4	3.4	0.76	0.75	0.75	4.5	4.6	4.5
9	7.59	7.84	7.71	31.0	29.9	30.0	8.6	8.3	8.4	36.3	34.5	35.4	3.6	3.6	3.6	0.85	0.87	0.86	4.2	4.2	4.2
10	7.36	7.38	7.37	32.0	32.2	32.1	10.3	9.8	10.0	41.4	39.4	40.4	3.1	3.3	3.2	0.77	0.82	0.79	4.0	4.0	4.0
11	8.04	7.10	7.57	29.7	33.9	31.8	8.2	9.5	8.8	35.4	40.6	38.0	3.6	3.6	3.6	0.84	0.83	0.84	4.3	4.3	4.3
12	6.75	7.49	7.12	33.7	32.0	32.8	9.0	9.0	9.0	35.4	36.4	35.9	3.7	3.6	3.7	0.95	0.88	0.91	3.9	4.1	4.0
13	7.06	7.08	7.17	31.0	31.8	31.4	9.3	9.3	9.3	32.7	32.6	32.7	3.3	3.4	3.3	0.94	0.98	0.96	3.5	3.5	3.5
14	6.61	6.65	6.63	36.8	36.4	36.6	10.3	10.8	10.6	27.2	28.1	27.6	3.6	3.4	3.5	1.35	1.29	1.32	2.6	2.6	2.6
15	6.87	6.88	6.87	33.2	33.2	33.2	8.7	8.2	8.4	26.4	27.2	26.8	3.7	4.1	3.9	1.26	1.22	1.24	3.0	3.3	3.1
16	6.88	6.86	6.87	32.4	32.2	32.3	9.7	9.3	9.5	26.6	25.6	26.1	3.3	3.5	3.4	1.22	1.25	1.24	2.7	2.8	2.7
17	7.86	7.87	7.87	28.0	29.5	28.7	8.4	8.9	8.6	37.0	35.1	36.0	3.3	3.3	3.3	0.76	0.84	0.80	4.4	3.9	4.1
18	7.16	7.78	7.47	29.3	29.2	29.2	8.2	8.0	8.1	31.3	28.1	29.7	3.6	3.6	3.6	0.94	1.04	0.99	3.8	3.5	3.6
19	7.16	7.29	7.22	31.3	31.4	31.3	9.9	9.6	9.7	38.9	37.5	38.2	3.2	3.3	3.2	0.80	0.84	0.82	3.9	3.9	3.9
20	7.34	7.37	7.35	31.0	31.8	31.4	8.3	8.0	8.1	34.1	32.0	33.0	3.8	4.0	3.9	0.91	0.99	0.95	4.1	4.0	4.1
21	6.98	6.84	6.91	30.1	30.1	30.1	8.7	9.5	9.1	34.8	36.7	35.7	3.5	3.2	3.4	0.87	0.82	0.84	4.0	3.9	3.9
22	6.59	6.57	6.58	32.0	32.0	32.0	9.4	9.6	9.5	38.1	36.7	37.4	3.4	3.3	3.4	0.84	0.87	0.85	4.1	3.8	3.9
23	6.78	7.51	7.13	31.9	30.4	31.1	9.7	8.8	9.2	35.7	32.5	34.1	3.3	3.5	3.4	0.89	0.94	0.92	3.7	3.7	3.7
24	6.49	6.58	6.54	35.4	34.2	34.8	11.1	10.9	11.0	31.4	32.8	32.1	3.2	3.1	3.1	1.13	1.04	1.09	2.8	3.0	2.9
25	7.46	7.35	7.40	29.7	28.3	29.0	8.9	8.9	8.9	37.0	36.3	36.7	3.3	3.2	3.3	0.80	0.78	0.79	4.2	4.1	4.1
26	7.86	7.91	7.89	29.4	29.7	29.6	9.6	9.4	9.5	36.0	35.3	35.7	3.1	3.2	3.2	0.82	0.84	0.83	3.8	3.8	3.8
27	7.88	7.89	7.89	30.2	29.4	29.8	8.6	8.1	8.3	36.3	35.2	35.8	3.5	3.6	3.6	0.83	0.84	0.83	4.2	4.4	4.3
28	7.19	6.99	7.09	30.7	30.3	30.5	9.0	8.6	8.8	32.4	32.9	32.7	3.4	3.5	3.5	0.95	0.93	0.94	3.6	3.8	3.7
29	7.54	7.29	7.41	31.3	29.1	30.2	9.3	9.4	9.3	35.0	35.8	35.4	3.4	3.1	3.3	0.89	0.81	0.85	3.8	3.8	3.8
30	7.25	7.24	7.25	32.1	31.8	32.0	10.2	10.2	10.2	36.7	37.0	36.9	3.2	3.1	3.2	0.87	0.86	0.86	3.6	3.6	3.6
31	7.29	7.40	7.34	28.4	28.2	28.3	8.4	8.4	8.4	32.5	33.0	32.8	3.4	3.4	3.4	0.87	0.85	0.86	3.9	3.9	3.9
32	7.54	7.12	7.33	27.6	28.5	28.0	8.0	8.8	8.4	32.9	34.7	33.8	3.6	3.2	3.4	0.84	0.82	0.83	4.3	3.9	4.1

Fig. I.—Plan of Field Manurial Experiment (4×4 Latin Square), from which Leaf Samples were taken.

NK 8	NPK 7	K 9	PK 10	NP 24	N 23	O 25	P 26
PK 5	K 6	NPK 12	NK 11	O 21	P 22	NP 28	N 27
P 4	O 3	N 13	NP 14	K 20	PK 19	NPK 29	NK 30
N 1	NP 2	P 16	O 15	NK 17	NPK 18	PK 32	K 31

Main Plot Treatments ; O, N, K, NK.

Half-Plot Treatment ; P. Leaf Sample Numbers 1, 2, &c.

Table II.

Test of Accuracy of Sampling : Analysis of Variance between Plots Compared with Variance between Duplicates.

Due to	D.F.	Sum of Squares	Variance V	Log_e	$\frac{1}{2}\text{Log}_e$	Z
Total	63	39787	—	—	—	—
Plots	31	38905	1255	7.1349	3.5674	1.9085
Duplicates (Error)....	32	882	27.6	3.3178	1.6589	—

Observed value of "Z" (1 : 100 point)=0.4519 *i.e.* Variation between duplicate samples is negligible compared with variation between plots.

(*b*) *Discussion of Results.*

One of the most important aims of this investigation is to provide an accurate test of the method of leaf sampling which has been adopted. The most suitable stage of growth and type of leaf material has been established by previous investigations, but it is of great importance that conclusive evidence should be obtained as to whether the leaf composition of a group of cacao trees is accurately indicated by the composition of a small random sample of leaves. The results presented in Table II show that the variation in composition between duplicate samples is negligible compared with the variation between different plots. Strictly speaking, these results refer only to the nitrogen/potash ratio of the leaf, but inspection of the data in Table I shows that for all the different leaf components, and for all the nutrient ratios, determinations on duplicate samples agree very closely. The method of sampling adopted is therefore adequate for the purposes of this investigation, and the composition of the individual leaf samples is truly representative of the group of trees from which they were taken.

II.—Relationship Between Leaf Composition and Yield of Cacao trees.

(*a*) *Experimental Data.*

Correlation coefficients have been calculated between the different leaf components and yield for all the different plots in the experiment. The results are set out in Table III.

Table III.

Relationship between Composition of Leaves and Yield of Cacao : Values of Correlation Coefficients (r)

Index of Leaf Composition.	Correlation (r) with Yield.	
	Weight of Wet Cacao.	No. of Pods.
Ash % Dry Matter	+0.583	+0.522
N % Dry Matter	−0.234	−0.353
N % Ash	−0.646	−0.672
P_2O_5 % Dry Matter	−0.055	−0.192
P_2O_5 % Ash	−0.483	−0.575
K_2O % Dry Matter	+0.578	+0.441
K_2O % Ash	+0.425	+0.363
N/P_2O_5 Ratio	+0.469	+0.538
N/K_2O Ratio	−0.634	−0.573
K_2O/P_2O_5 Ratio	+0.533	+0.513

Any value of "r" exceeding 0.409 shows a significant relationship with a minimum probability of 50 : 1.

(*b*) *Discussion of Results.*

The results in Table III show that, at this site, *there is a remarkably close relationship between the chemical composition of the leaf and quantitative yield of cacao.* Significant positive correlations obtain between (*a*) total ash content of the leaf and yield, (*b*) potash/phosphate ratio and yield, and (*c*) nitrogen/phosphate ratio and yield, whilst a significant negative correlation obtains between (*d*) nitrogen/potash ratio and yield. High yielding capacity of the cacao tree is evidently associated with a high total ash content of the leaf, a low nitrogen/potash ratio, a high potash/phosphate ratio and a high nitrogen/phosphate ratio. The optimum numerical values for these four main indices of leaf composition appear likely to be of the same order as those previously reported for the Standard Good Plot, namely, (1) total ash 9.9 per cent., (2) N/K_2O, 0.89, (3) K_2O/P_2O_5, 5.21, (4) N/P_2O_5, 4.66.

The figures in Table III show that significant correlations also obtain between the individual leaf components and yield, *i.e.*, increased yields are associated with an increased potash content, and a decreased nitrogen and phosphate content of the leaf. As an index of the nutrient requirements of the cacao tree, however, the nutrient ratios are probably of greater value, since these ratios take into account the relative as well as the absolute amounts of the different elements present in the leaf.

Leaf samples taken from the *control plots* of this experiment show a low percentage of ash in dry matter, a high nitrogen/potash ratio, a low potash/phosphate ratio, and a low nitrogen/phosphate ratio in the leaf compared with the standard optimum values. In order to determine whether a particular manurial treatment has resulted in a more balanced nutrient supply, it will be necessary to study the effect of each treatment on these four main leaf index values. If a particular manurial treatment has so altered the composition of the leaf that the nutrient ratio values approach more closely to the standard optimum values, then it may be assumed that the treatment has been beneficial, and has resulted in a more balanced nutrient supply to the cacao tree. On the other hand, if a particular manurial treatment has produced the reverse effect on the four main leaf index values, then it may be assumed that the treatment is harmful, and has caused the nutrient supply to become even more unbalanced than that of the control plots.

(III)—The Effect of Manurial Treatments on the Chemical Composition of the Cacao Tree.

(*a*) *Experimental Data.*

The values for each individual leaf component, as well as for the different nutrient ratios, have been subjected to detailed statistical examination by means of Fishes's Analysis of Variance method. From considerations of space, the complete tables of statistics are omitted from the text of this article, but the results are summarised in Tables VI and VII. In order further to illustrate the definite significant effects of the manurial treatments on leaf composition, a full analysis of variance is presented in Tables IV and V, showing the effects of the different treatments on the nitrogen/potash ratio and the potash/phosphate ratio of the leaf respectively.

Table IV.

Effect of Manurial Treatments on the Nitrogen/Potash Ratio of Cacao Leaves : Analysis of Variance.

Due To	D.F.	S.S.	V.	$\text{Log}_{e.}$	$\frac{1}{2}\text{Log}_{e.}$	Calculated "Z"	Observed "Z" 1 : 20
TOTAL MAIN PLOTS	15	7394	—	—	—	—	—
Rows	3	2434	—	—	—	—	—
Columns	3	1604	—	—	—	—	—
MAIN PLOT TREATMENTS							
Total	3	2934	978	6.8855	3.4427	1.3163	0.7798
K	1	2888	2888	7.9684	3.9842	1.8578	0.8948
N....	1	45	45	—	—	—	—
Interaction N×K	1	1	1	—	—	—	—
ERROR (*a*)	6	422	70.3	4.2528	2.1264	—	—
TOTAL SUB-PLOTS	31	9410	—	—	—	—	—
Sub-Plot Treatments	1	181	181	5.1985	2.5992	0.6462	0.7788
SUB-PLOT INTERACTIONS.							
Total	3	1238	413	6.0235	3.0117	1.0587	0.6250
P×N	1	1058	1058	6.9642	3.4821	1.5291	0.7788
P×K	1	136	136	4.9127	2.4563	0.5033	0.7788
P×N×K	1	44	44	—	—	—	—
ERROR (*b*)	12	597	49.7	3.9060	1.9530	—	—

In order to demonstrate a significant effect due to a particular treatment, the calculated value of "Z" must exceed the observed value of "Z".

Table V.

Effect of Manurial Treatments on the Potash/Phosphate Ratio in Cacao Leaves : Analysis of Variance.

Due To	D.F.	S.S	V.	$\text{Log}_{e.}$	$\frac{1}{2}\text{Log}_{e.}$	Calculated "Z"	Observed "Z" 1 : 20
TOTAL MAIN PLOTS	15	930	—	—	—	—	—
Rows	3	355	—	—	—	—	—
Columns	3	56	—	—	—	—	—
MAIN PLOT TREATMENTS							
Total	3	454	151	5.0173	2.5086	1.3097	0.7798
K	1	442	442	6.0913	3.0456	1.8467	0.8948
N	1	9	—	—	—	—	—
Interaction N×K	1	3	—	—	—	—	—
ERROR (*a*)	6	65	11	2.3979	1.1989	—	—
TOTAL SUB-PLOTS	31	1127	—	—	—	—	—
Sub-Plot Treatments	1	34	34	3.5264	1.7632	0.8351	0.7788
SUB-PLOT INTERACTIONS.							
Total	3	86	29	3.3673	1.6836	0.7555	0.6250
P×N	1	52	52	3.9513	1.9756	1.0475	0.7788
P×K	1	30	30	3.4012	1.7006	0.7725	0.7788
P×N×K	1	4	4	—	—	—	—
ERROR (*b*)	12	77	6.4	1.8563	0.9281	—	—

In order to demonstrate a significant effect due to a particular treatment, the calculated value of "Z" must exceed the observed value of "Z".

Table VI.

Effect of Manurial Treatments on Composition of Cacao Leaves: Mean Composition of Leaves from Differently Treated Plots.

Manurial Treatments.	% Ash in Dry Matter	Composition of Ash.			Nutrient Ratios.		
		N %	P_2O_5 %	K_2O %	N/P_2O_5.	N/K_2O	K_2O/P_2O_5
Control	6.73	33.0	9.43	32.2	3.52	1.05	3.43
N	7.10	31.5	9.15	33.8	3.42	0.94	3.70
P	7.05	32.3	9.80	32.8	3.32	1.00	3.35
K	7.48	29.5	8.18	33.8	3.68	0.88	4.10
NP	6.70	34.5	10.13	30.0	3.40	1.16	2.97
NK	7.58	30.3	8.93	37.0	3.40	0.81	4.12
PK	7.63	29.3	8.70	36.8	3.39	0.80	4.28
NPK	7.45	30.8	8.63	34.0	3.60	0.91	3.93
Standard Error of Difference Between Means	0.26	1.16	0.46	1.0	0.15	0.06	0.18
S.E. × " t "	0.57	2.53	1.00	2.18	0.33	0.12	0.39

Each value is the mean of four plots. Value of " t " equals 2.18, and the Standard Error multiplied by " t " equals the amount by which two means must differ in order that the difference may be significant with a minimum probability of 20 : 1.

Table VII.

Effect of Manurial Treatments on Composition of Cacao Leaves. Differences in Mean Composition of Leaves from Differently Treated Plots.

Manurial Treatments.	% Ash in Dry Matter	Composition of Ash.			Nutrient Ratios.		
		N %	P_2O_5 %	K_2O %	N/P_2O_5	N/K_2O	K_2O/P_2O_5
Control	0	0	0	0	0		0
N	+0.37	−1.5	−0.28	+1.6	−0.10	−0.11	−0.27
P	+0.32	−0.7	+0.37	+0.6	−0.20	−0.05	−0.08
K	**+0.75**	**−3.5**	**−1.25**	+1.6	+0.16	**−0.17**	**+0.67**
NP	−0.03	+1.5	+0.70	−2.2	−0.12	+0.11	−0.46
NK	**+0.85**	**−2.7**	−0.50	**+4.8**	−0.12	**−0.24**	**+0.69**
PK	**+0.90**	**−3.7**	−0.73	**+4.6**	−0.13	**−0.25**	**+0.85**
NPK	**+0.72**	−2.2	−0.80	+1.8	+0.08	**−0.14**	**+0.50**
Standard Error of Difference Between two Means	0.26	1.16	0.46	1.0	0.15	0.06	0.18
S.E. × " t " = Difference Required for Significance	0.57	2.53	1.00	2.18	0.33	0.12	0.39

Values represent mean differences on leaf composition due to each manurial treatment. Differences must be greater than 2.18 times the Standard Error in order to be significant with a minimum probability of 20 : 1.

Differences which are significant are indicated by thicker type.

(*b*) *Discussion of Results.*

The results in Tables IV and V show that the potash treatments have been mainly effective in altering the composition of the leaf. A significant interaction between phosphate and nitrogen has also occurred, and this has caused a definite alteration in leaf composition. The effects of the different manurial treatments are shown in more detail by the summarised results in Tables VI and VII.

These results reveal the following significant effects of manurial treatments on leaf composition :—

(1) Potash alone has (*a*) increased the total ash, (*b*) decreased the nitrogen content of the leaf, (*c*) decreased the phosphate content of the ash, (*d*) decreased the nitrogen/potash ratio, and (*e*) increased the potash/phosphate ratio. All these effects have resulted in nutrient ratios more closely approximating to the standard optimum values, demonstrating that the application of potash manure alone has caused a much more evenly balanced nutrient supply to the cacao tree. Potash alone has produced no effect on the nitrogen/phosphate ratio, since the uptake of nitrogen and phosphate has decreased in equal proportion.

(2) Nitrogen alone has produced no definite effect on leaf composition.

(3) Phosphate alone has produced no definite effect on leaf composition.

(4) Phosphate applied together with potash has produced an additional beneficial effect, in that potash uptake has been increased.

(5) Nitrogen applied with potash has given a similar beneficial effect in that it has increased the potash uptake.

(6) Treatments including nitrogen together with phosphate resulted in an increased uptake of nitrogen and phosphate relative to potash. The application of nitrogen together with phosphate, has evidently produced a very unbalanced nutrient supply. When these two elements are applied together in the absence of potash, the composition of the leaf is so altered that it differs more widely from the standard optimum values.

The practical interpretation of these results is that *potash is markedly deficient in the soil at this site,* and is the limiting factor to growth and yield. Nitrogen and phosphate appear to be present in the soil in adequate quantities relative to potash. Beneficial effects have resulted only from potash treatments, but there is evidence that additional benefit might be derived from *small* dressings of nitrogen and phosphate applied together with potash. The existing nitrogen and phosphate treatments appear to be excessive relative to the potash supply. These findings closely agree with the results previously obtained by Pound concerning the effect of the various manurial treatments on yield.

General Discussion.

(1) *Leaf Analysis as a Means of Diagnosing Soil and Crop Nutrient Requirements.*

It is evident that the chemical composition of cacao leaves provides reliable information regarding prevailing nutrient supply of the cacao tree. There appears to be a close relationship between leaf composition and yielding capacity. The leaves of high yielding trees have a high total ash content, a high potash content relative to nitrogen and phosphate, and a low phosphate content relative to nitrogen and potash. Leaf composition may be best represented by four main indices, the optimum numerical values of which are approximately as follows :—(1) total ash, 9.9 per cent. of dry matter, (2) nitrogen/potash ratio, 0.89, (3) nitrogen/phosphate ratio, 4.66 and (4) potash/phosphate ratio, 5.21. This standard leaf composition indicates an adequate and balanced nutrient supply, resulting in maximum yield of mature cacao trees.

The effect of an unbalanced soil nutrient supply in altering leaf composition has now been investigated at two contrasted sites, namely, (1) where soil phosphate was markedly deficient, (2) where soil potash was markedly deficient. In both cases, the lack of nutrient balance in the soil is clearly indicated by the chemical composition of the leaf, which differs widely from that of the optimum standard. Phosphate deficiency or potash deficiency in the soil produce very similar effects on leaf composition. In both cases, the potash content of the leaf is decreased with regard to phosphate and nitrogen. In the case of potash deficiency, however, the nitrogen/phosphate ratio of the leaf is lower than in the case of phosphate deficiency. The distinction between the two different conditions is not very clearly indicated by the nitrogen/phosphate and potash content of the leaf alone, and it is hoped that the additional analyses for calcium, magnesium, iron, sulphate and chloride may afford further information.

From these results, a number of generalisations concerning the mineral nutrition of the cacao tree may be deduced. If one of the three main nutrient elements is deficient in the soil, the application of either of the other two nutrient elements alone will be ineffective, and in combination they may even produce positively harmful effects on growth and yield. Leaf analysis has shown that mature cacao trees require adequate supplies of potash for greatest growth and yield, high yield being associated with a high potash content of the leaf relative to nitrogen and phosphate. A shortage of potash may occur as a result of soil *phosphate* deficiency or soil *potash* deficiency. When conditions are such that potash uptake is adequate, then additional benefit to the cacao tree may be derived from added nitrogen, but a large nitrogen uptake relative to potash is definitely harmful. Phosphate appears to be required by mature cacao trees *in small quantities only* relative to potash and nitrogen.

Phosphate appears to be required only in such quantities as will ensure an adequate uptake of potash by the cacao tree. If available soil phosphate is present in excess, a luxury consumption of phosphate occurs, and the increased

phosphate uptake is associated with decreased yields. This is contrary to the findings of Carmago with regard to phosphate uptake of coffee plants. He showed by means of pot cultures that phosphate could be absorbed in considerable excess without noticeably affecting the growth of young coffee plants. In another section of this report, the suggestion is put forward that the cacao tree may be particularly sensitive to iron deficiency, and increased uptake of phosphate may therefore depress growth and yields, in that it interferes with the uptake and utilisation of iron. The additional chemical analyses which are being carried out on the present series of leaf samples should provide a reliable test of the accuracy of this hypothesis.

(2) *The Manurial Requirements of Cacao as Indicated by Soil Surveys, Field Manurial Experiments and Leaf Analysis.*

The results recently obtained by the chemical analysis of cacao leaves show that the correct manuring of the cacao crop must be considered from two different aspects, (*a*) the nutrient supplying ability and nutrient balance of the soil, and (*b*) the particular nutrient requirements of the cacao tree itself.

With regard to soil nutrient requirements, soil surveys have shown that phosphate deficiency is of chief importance in West Indian cacao soils. The deficiency of phosphate is much more marked and much more widespread than deficiency of potash, while nitrogen appears to be generally present in these soils in adequate amounts relative to phosphate and potash. Furthermore, field experiments have shown that very large increases in yield of mature cacao trees can be obtained by the application of phosphatic manures alone on a number of different soil types. With regard to the nutrient requirements of mature cacao trees, leaf analysis has shown that an adequate uptake of potash is of chief importance, while phosphate is required in small quantities only, an increased uptake of phosphate being associated with decreased yields.

At first sight these findings appear to be opposed to one another, but leaf analysis has provided the connecting link between these apparently contradictory results. Where soil *phosphate* is markedly deficient, the cacao tree is unable to obtain adequate supplies of *potash*. For such phosphate-deficient soils, the addition of potash alone is ineffective, but the application of phosphate alone, or phosphate together with potash, is beneficial, in that the uptake of potash is increased. If, however, phosphate is applied in excessive quantities, so that available phosphate is present in excess in the soil, a luxury consumption of phosphate occurs, and this exerts definitely harmful effects on the nutrient balance within the tree and depresses yields. Phosphate manuring on cacao soils should therefore be carried out with caution, even though soil analysis may indicate a marked deficiency of phosphate. Field experiments have shown that there is little actual danger of excessive application of phosphate on acidic, non-gypseous heavy clay soils, since the added phosphate is fixed in an insoluble and difficultly available form. On phosphate-deficient *sandy* soils, however, most benefit was derived from moderate applications of phosphate, while heavy dressings were shown to be definitely excessive. Furthermore, leaf analysis has indicated that, once soil phosphate-deficiency has been remedied, further benefit should be derived from the application of potash manures. Indeed potash manuring will have to be carried out if high yields are to be maintained. Field experiments recently laid down should provide more conclusive evidence on this point.

To summarise these findings, it may be said that the most important nutrient requirement for high yield of mature cacao trees is an adequate supply of *potash*. Where soil *phosphate* is deficient, however, the cacao tree is unable to obtain adequate supplies of potash, even though potash be present in the soil in adequate amount. Since phosphate-deficiency is much more marked and much more widespread than potash deficiency in West Indian cacao soils, the most urgent general manurial need in these areas is phosphate manuring. Excessive phosphate supply is definitely harmful, and phosphate manuring must therefore be carried out with caution, particularly on sandy soils and other soils where added phosphate may be present in an easily soluble form. Once soil phosphate deficiency has been remedied, additional benefit may be derived from potash, and possibly nitrogen manuring; indeed the aim of phosphate manuring on phosphate-deficient soils is to obtain a nutrient balance in the soil so that the cacao tree is able to obtain adequate supplies of potash and nitrogen. The general results of this investigation have emphasized the great importance of a precise knowledge of soil conditions. The cacao tree is evidently very sensitive to correct nutrient balance in the soil, and the application of haphazard or arbitrary manurial mixtures will be wasteful or even definitely harmful. The recommendations outlined above, and contained in other sections of this report, are generally applicable to cacao growing areas in the West Indies, but, for other cacao growing areas, these recommendations may have to be modified depending upon soil conditions. For full benefit to be obtained, manurial substances must be applied in particular and specific ratios, depending upon the specific requirements of the soil in each particular area. In order to obtain precise knowledge of these requirements, soil surveys based on laboratory investigations should first be prosecuted. The different soil-types thereby delineated should next be tested by carefully-planned field manurial experiments, and the yield results finally checked by chemical analysis of representative leaf material.

Summary

(1) The possibility of diagnosing soil and nutrient requirements of cacao by means of chemical analysis of leaves has been studied. The present investigation forms a continuation of other earlier investigations, the results of which have been previously reported.

(2) Leaf samples were collected from a complex field manurial experiment, involving the use of nitrogen, phosphate and potash treatments, both alone and in combination. The soil of this experimental site is markedly deficient in *potash*, in contrast to the soil at the site of previous investigations, which was markedly deficient in *phosphate*.

(3) Duplicate leaf samples were taken from 32 separate plots, and analysed in the laboratory for total ash, nitrogen, potash and phosphate contents.

(4) The analytical data have been subjected to detailed statistical examination, and the following definite results have been obtained.

(*a*) The variation in composition between duplicate samples is negligible as compared with the variation between different plots. The method of sampling is therefore adequate for the purposes of the investigation, and the chemical composition of a small random sample of young cacao leaves is truly representative of the group of trees from which it is taken.

(*b*) A close relationship exists between leaf composition and quantitative yield of individual plots. High yield is associated with a high potash content of the leaf relative to nitrogen and phosphate, and a low phosphate content of the leaf relative to nitrogen and potash.

(*c*) As an index of nutrient supply, the chemical composition of cacao leaves may be best represented by four main factors, the optimum numerical values of which are approximately, (1) total ash, 9.9 per cent. of dry matter, (2) nitrogen/potash ratio, 0.89, (3) nitrogen/phosphate ratio, 4.66 and (4) potash/phosphate ratio, 5.21. This standard leaf composition indicates an adequate and balanced nutrient supply, resulting in maximum yield of mature cacao trees.

(*d*) The chemical composition of leaf samples taken from the control plots receiving no manurial treatments, differed widely from the standard optimum values.

(*e*) Potash manures have definitely altered the composition of the leaf, so that it approaches much more closely to the standard optimum values.

(*f*) Nitrogen alone and phosphate alone have had no definite effect on leaf composition, but nitrogen and phosphate applied together have definitely altered leaf composition, so that it diverges more widely from the standard optimum values.

(*g*) Changes in leaf composition show that potash manure alone has been beneficial, and has resulted in a more balanced nutrient supply to the trees. Nitrogen alone and phosphate alone have been ineffective, but these two elements applied in combination have caused the nutrient supply to become even more unbalanced than that of the control plots. The effects of manurial treatments on leaf composition closely agree with the effects of manurial treatments on yield of cacao.

(5) The results obtained for cacao growing on potash deficient soil have been compared with results of previous investigations of cacao growing on an experimental site where *phosphate* is markedly deficient in the soil.

(6) The collected results have shown that mature cacao trees require adequate supplies of potash for greatest growth and yield. A shortage of potash in the plant may occur as a result of soil *phosphate* deficiency or soil *potash* deficiency, the former condition being of much more widespread occurrence in West Indian cacao growing areas.

(7) Leaf analysis has proved of great value in providing a more exact interpretation of the results of soil surveys and field experiments.

Acknowledgment.

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PART V.

SOME EFFECTS OF DEFICIENCIES OF ESSENTIAL NUTRIENT ELEMENTS ON THE GROWTH OF YOUNG CACAO PLANTS.

By J. A. McDonald.
(*Chemist for Cacao Research.*)

Introduction.

Many investigations have been conducted to determine the effects of deficiency of various nutrient elements on the growth of temperate fruit trees. Outstanding among these investigations are the experiments carried out by Wallace at Long Ashton, England. Wallace showed, by means of sand cultures, that deficiency of nitrogen, phosphorus, potassium, calcium or magnesium produced distinctive symptoms and growth characteristics in fruit trees. The knowledge gained from these sand culture experiments has proved of considerable practical value in the cultivation of fruit trees in northern countries. It has often been possible to diagnose the deficiency or specific nutrient elements in the field by identifying the foliar symptoms and growth characteristics associated with deficiency of that element in sand culture experiments. In the Third Annual Report on Cacao Research, the observed effects of nutrient deficiencies on temperate fruit trees were discussed in detail, and the possible application of some of these findings to problems concerning the nutrition of the cacao tree was indicated.

At the beginning of 1934, two series of sand culture experiments were initiated at the College. Series I was a preliminary experiment, in which cacao seedlings were grown in sand culture in small flower pots. These sand cultures were initiated to obtain preliminary evidence, in as short a time as possible, of the foliar symptoms and growth characteristics of young cacao plants, associated with deficiencies of nitrogen, phosphorus and potassium in the nutrient solution. Series II is a similar trial of the effects of these deficiencies on a large scale, the cacao plants consisting of vegetatively-propagated material of homogeneous genetic constitution, supplied by the Botanical Section of Cacao Research. This investigation differs from Series I in that the cacao plants are being grown in large tubs of sand, so that it will be possible to continue the experiment for a longer period of time. The Series I experiments had to be discontinued when the cacao seedlings were ten months old, since some of the plants had then reached a size when their growth was restricted by the inadequate size of the pots. The results obtained in Series I experiments alone are presented in this article.

Experimental.

Cacao seeds were planted in pure quartz sand in 16 earthenware flower pots, each pot containing about three and a half kilograms of sand. The seeds for planting were obtained from two pods taken from the same tree. The sand was identical with that used by Wallace at Long Ashton, and was specially obtained from England. Three seeds were planted in each pot, thus intentionally producing a crowded root environment, since one of the main objects of the investigation was to obtain results quickly. By this means, any minute quantities of the deficient element present in the sand, the material of the flower pot, or the seeds would be quickly exhausted. Distilled water was used throughout the experiment for watering the plants and for making the nutrient solutions.

The 16 pot cultures were divided into four groups of four each, as follows :—(A) complete nutrient, (B) lacking nitrogen, (C) lacking potassium, and (D) lacking phosphorus. The "complete nutrient" solution had the following composition :—

Ammonium chloride	76.5 grm.
Potassium nitrate	120.0 ,,
Calcium nitrate	28.0 ,,
Potassium Phosphate (K_2HPO_4)	20.0 ,,
Calcium sulphate	40.0 ,,
Magnesium sulphate	60.0 ,,
Sodium chloride	10.0 ,,
Ferric chloride	8.0 ,,
Distilled water	2000 cc.

10 cc. of this stock solution were diluted to one litre for application to the plants.

In order to derive the various "omission solutions", the salt (or salts) containing the element to be omitted was replaced by the sodium salt in place of potassium, or the chloride in place of nitrate or phosphate. The nutrient solutions were applied to the plants twice every week at the rate of 500 cc. dilute solution per pot. In order to guard against deficiency of certain rare elements, an additional solution of the following composition was applied to all the plants once every month.

Manganese sulphate	0.76 grm.
Manganese chloride	0.58 ,,
Aluminium sulphate	1.46 ,,
Boric acid	0.72 ,,
Zinc sulphate	1.10 ,,
Distilled water	10 litres.

40 cc. of this stock solution was diluted to one litre for application to the plants.

Throughout the duration of the experiment, observations were made on the growth features of the plants. After ten months' growth, photographs were taken of the plants in one pot typical of each group. Measurements were also made of the height and stem diameter of the two largest plants in each pot. The plants were then removed from the sand, and the dry weight of plant material from each pot was determined.

Results.

Data showing the amount of growth attained by the plants in each group are presented in Table I. Plate III, Figs. 1 and 2 further show the growth of the plants and the comparative appearance after seven months' and ten months' growth respectively.

(1) *Nitrogen Omitted Group.*

For the first three months after planting, these plants made normal growth, compared with the complete nutrient series. During the fourth month, the leaves of the nitrogen-deficient plants were noticeably paler green in colour, and growth was retarded. Subsequently, the difference in size became more marked, and the pale yellowish-green colour of the leaves became more accentuated. The comparative appearance of the plants after seven months' growth is shown in Fig. 1. From seven to ten months, the nitrogen-deficient plants made very little growth; this is clearly shown by comparing Figs. 1 and 2 and the data in Table I. The final appearance of the nitrogen-deficient plants may be described thus: Growth was very stunted. Leaves were small and pale yellowish-green in colour. They were few in number, and occurred only close to the growing point, giving the plants a characteristic bare-wood appearance. Development of the root system was normal in proportion to the development of stem and leaves. These symptoms in many respects are similar to those described by Wallace for nitrogen-deficient temperate fruit trees.

(2) *Potassium Ommitted Group.*

The comparative appearance of the plants after seven months' growth is shown in Fig. 1. Up to this time, the potassium-deficient plants showed normal growth and appearance. During the eighth month, one of the four potassium-deficient cultures began to show leaf-scorch symptoms or marginal browning of the leaves. At the end of the ninth month, all four cultures were showing leaf-scorch symptoms, which became progressively worse until the end of the experiment. The comparative appearance of the plants is shown in Fig. 2. The typical leaf-scorch symptoms are shown more clearly in Plate IV, Figs. 3 and 4. This typical lesion of cacao leaves, due to potassium deficiency, begins as a greyish-brown discolouration of the edges of the leaves, generally near the leaf tip. In later stages, the browning of the leaf extends upwards along the leaf edges towards the petiole, and inwards between the veins as far as the mid-rib. In Fig. 4, many leaves are shown in which the condition is well developed. The data in Table I show that, during the experimental period of ten months, there has been no marked difference in the amount of growth between the potassium-deficient plants and complete nutrient plants. The average height of the plants in both groups is similar, but the stem diameter of the potassium-deficient plants is less, *i.e.*, they have made a more spindly growth. The rather whip-like appearance of the stems is shown in Fig. 2. Total root development appears to be unaffected by potassium deficiency, but the proportion of fine roots is greater than in the complete nutrient cultures.

(3) *Phosphate Omitted Group.*

The data in Table I and Figs. 1 and 2 show that, after ten months' growth, the phosphate-deficient plants are nearly normal in growth and appearance. No very marked foliar symptoms or growth characteristics, due to phosphate deficiency, can be observed. Certain small differences in growth and appearance, however, are worth recording. The leaves are small, though otherwise healthy and normal in appearance. Height growth is slightly reduced, but the diameter of the stem is normal, so that the plants exhibit a short and sturdy appearance as compared with potassium-deficient plants. Total root development is normal, but the proportionate amount of fine roots is considerably less than in the complete nutrient cultures.

General Conclusions.

Sand culture experiments with cacao seedlings have demonstrated definite foliar symptoms and growth characteristics due to nitrogen deficiency and potassium deficiency respectively. During the growth period of the experiment (ten months), only small differences in growth due to phosphate deficiency were observed. The practical value of these findings lies in the possibility of diagnosing nutrient deficiencies in the field merely by observing growth characteristics or foliar symptoms similar to those obtained in the sand cultures. The recorded symptoms of nitrogen deficiency and potassium deficiency are very distinctive and easily recognised; they should be particularly useful for field diagnostic purposes. Foliar symptoms of potassium deficiency, similar to those obtained in sand culture, have recently been observed in cacao trees growing in the Diego Martin Valley, North Trinidad, which is an area where soil potash is known to be deficient.

Summary.

(1) An investigation has been made of the effect of deficiencies of nitrogen, potassium and phosphorus on cacao plants growing in sand cultures.

(2) Nitrogen deficiency in young cacao plants is characterised by very stunted growth and a pale yellowish-green colour of the leaves. The leaves are small in size and few in number, and occur only at the end of the stems, near the growing point, giving the plants a characteristic bare-wood appearance. The development of the root system is stunted and proportional to the shoot and leaf growth. The proportional development of coarse and fine roots is normal.

(3) Potassium deficiency in young cacao plants is characterised by a marginal browning of the leaf, similar to the leaf-scorch symptoms due to potassium deficiency in temperate fruit trees,

FIG. 1.—*Effect of Deficiency of Nitrogen, Potassium and Phosphorus on the Growth of Cacao Seedlings. Seven months growth.*

A.—Complete Nutrient. *B.—Lacking Nitrogen.*
C.—Lacking Potassium. *D.—Lacking Phosphorus.*

A. B. C. D.

FIG. 2.—*Effect of Deficiency of Nitrogen, Potassium and Phosphorus on the Growth of Cacao Seedlings. Ten months growth.*

A.—Complete Nutrient. *B.—Lacking Nitrogen.*
C.—Lacking Potassium. *D.—Lacking Phosphorus.*

PLATE IV.

FIG. 3. — *Young Cacao Seedlings showing Marginal Leaf Scorch due to Potassium Deficiency.*

Fig. 4. — Cacao Leaves showing Marginal Leaf Scorch due to Potassium Deficiency.

In cacao leaves, the marginal browning commences at the edges of the leaf near to the tip. In later stages, the lesion spreads upwards along the edges, towards the petiole, and inwards between the veins, towards the mid-rib. The appearance of potassium-deficient cacao plants is tall and spindly. The height growth is normal, but the diameter of the stems is relatively small. Total root development is normal, but there is a larger proportional development of fine roots.

(4) Cacao plants growing in a phosphate-deficient nutrient solution were nearly normal in appearance after ten months' growth. The height of the phosphate-deficient plants, and the size of the leaves, were slightly subnormal, but the number of leaves and the diameter of the stems were normal. Total root development is normal but there is a smaller proportional development of fine roots.

(5) These preliminary experiments have yielded results which may be of great practical utility, since it may be possible to diagnose deficiency of nitrogen and of potassium under field conditions, merely by observation of foliar symptoms and growth characteristics of the cacao tree.

Acknowledgment.

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Table I.

Effects of Nitrogen, Potassium and Phosphorus Deficiency on the Growth of Cacao Seedlings: Growth Measurements.

	Five Months' Growth.		Ten Months' Growth.						
	Height cms.	Diameter mm.	Height cms.	Diameter mm.	Dry Wt. of Stem and Leaves.	Dry Wt. of Coarse Roots.	Dry Wt. of Fine Roots.	Total Dry Wt. of Roots.	Coarse Roots/ Fine Roots; Ratio.
Complete Nutrient.									
1	21.5	7.67	71.6	14.17	27.30	9.2	4.0	13.2	2.30
2	23.2	8.46	68.7	14.42	28.30	10.2	4.2	14.4	2.43
3	17.2	8.01	65.0	12.73	19.10	7.7	3.2	10.9	2.41
4	21.4	7.97	70.0	13.30	30.40	9.5	4.6	14.1	2.06
Means	20.8	8.03	68.8	13.66	26.28	9.2	4.0	13.2	2.30
Lacking Nitrogen.									
1	19.9	7.80	28.0	10.66	10.30	6.0	2.5	8.5	2.40
2	22.4	7.22	25.1	9.13	10.20	6.6	2.0	8.6	3.20
3	15.8	7.30	21.5	11.59	8.80	5.5	2.5	8.0	2.20
4	16.7	7.18	22.0	9.66	7.00	5.4	1.9	7.3	2.84
Means	18.7	7.38	24.2	10.26	9.08	5.9	2.2	8.1	2.68
Lacking Potassium.									
1	22.6	7.50	64.6	12.72	19.30	8.6	3.3	11.9	2.61
2	24.1	8.04	75.8	12.80	26.70	10.0	5.5	15.5	1.82
3	23.8	7.00	72.4	12.50	24.00	8.6	5.8	14.4	1.48
4	27.7	8.64	62.7	12.41	18.20	8.5	3.7	12.2	2.30
Means	24.5	7.79	68.9	12.61	22.1	8.9	4.6	13.5	1.94
Lacking Phosphorus.									
1	25.4	8.10	66.2	14.27	31.0	13.7	4.0	14.7	3.42
2	23.4	8.61	63.5	14.19	28.4	12.7	4.0	16.7	3.18
3	18.3	7.82	56.0	12.60	20.5	8.6	3.0	11.6	2.87
4	19.5	7.63	62.6	13.01	17.9	8.5	2.8	11.3	3.04
Means	21.6	8.04	62.1	13.52	24.5	10.9	3.4	13.6	3.20

PART VI.

PHOSPHATE FIXATION IN SOILS IN RELATION TO IRON AVAILABILITY, AND ITS POSSIBLE CONNECTION WITH THE GYPSUM PHOSPHATE PROBLEM IN CACAO SOILS.

By J. A. McDonald.
(*Chemist for Cacao Research*).

(1) Phosphate Fixation in Soils.

Recent investigations (1) have shown that certain soils have a high "fixing power" for phosphate, whereby added phosphate is retained in the surface layers of soil in a form unavailable to the plant. The fixation of phosphate is apparently determined partly by the reaction of the soil, and partly by the quantity and the nature of the bases present, either in the absorbed or the soluble condition.

In markedly acidic soils which possess low contents of exchangeable bases (potassium, calcium and magnesium), added phosphate may be fixed as iron and aluminium phosphates, which are relatively insoluble in water. In faintly acidic or neutral soils, in which there is a higher content of calcium compounds, added phosphate is fixed as calcium phosphate, which is relatively much more soluble and more readily available to plants. As the reaction of the soil becomes more alkaline, the solubility of calcium phosphate decreases. It would appear, therefore, that phosphate theoretically would be most easily and quickly available to the plant when added to acidic soils having an initial high content of available calcium. Acidity of the soil and a high calcium content seldom occur together, since an acidic reaction of the soil implies excessive leaching of bases. Gypseous clay soils in Trinidad often possess a markedly acidic reaction, and at the same time a high content of available calcium. Such soils should therefore fix added phosphate in an easily-soluble form, and readily available to the plant.

(2) Effect of Phosphate on Iron Availability.

Sideris and Kraus, in Hawaii, have shown (2) that the availability of iron to plants is closely related, both to the reaction, and to the phosphate content of the soil. Heavy applications of phosphatic manures caused a depression in the yield of pineapples on certain soils, due to the interference of phosphate with the solubility of iron and its availability to the plant. They concluded that phosphate, added to highly acidic soils, is fixed as iron and aluminium phosphates, which are relatively insoluble. From these insoluble compounds, both phosphate and iron may be obtained by the plants in small but sufficient quantities. For soils of faintly acidic or neutral reaction, on the other hand, added phosphate may be fixed as calcium phosphate, which is relatively more soluble, but nevertheless, still interferes with the solubility and availability of iron. In such cases, phosphatic manures may therefore depress the yields of certain crops which are particularly sensitive to iron deficiency.

(3) Gypsum-phosphate Problem in Cacao Soils.

Many cacao soils in Trinidad (for example, the Brasso Clay type) contain large amounts of available phosphate, but they normally produce poor crops. This behaviour is contrary to the general findings of soil surveys, and suggests that there must be some special reason for the high-phosphate-low-yield association. The soils have a high gypsum content, and the low yield may be due to the presence in the soil of phosphates in a form which interferes with the availability of iron to the cacao tree. Furthermore, certain other gypseous soils contain small amounts of available phosphate, yet they fail to respond to applications of phosphatic manures, probably for the same reason, namely the fixation of added phosphate in a form which depresses the availability of iron.

These observations suggest that the harmful effect of gypsum in cacao soils is closely associated with phosphate supply, the two factors in some way interfering with the availability of iron to the cacao tree. The researches of Sideris and Kraus indicate that the abundance of calcium, present in gypseous soils, forms phosphate compounds which interfere with the uptake and utilisation of iron. This hypothesis, namely, that abundant calcium, in the presence of phosphate, may depress the growth and yield of the cacao tree, is not supported, however, by the bulk of the evidence obtained by chemical examination of a very large number of cacao soils. Indeed, fertile cacao soils in the British West Indies usually possess a high calcium status, associated with an abundant supply of available soil phosphate, while the reaction of such soils varies over a wide range. A high gypsum content of the soil implies that sulphate as well as calcium are present in abundance, and it is suggested that a high concentration of sulphate may cause a lowered availability of iron in the presence of added phosphate.

Relevant evidence might be obtainable by determining the iron, sulphate and phosphate contents of cacao leaves taken from differently-treated plots in field manurial experiments. As a preliminary test of the value of this method, iron determinations were carried out on composite cacao leaf samples obtained in a field manurial experiment in which phosphate had

been applied, both alone and in combination with nitrogen and potash. The analytical results indicated that the application of phosphate alone caused no difference in the iron content of the leaves, but that, when phosphate was applied in combination with nitrogen or potash, there was a very marked decrease in the iron content of the leaves. The nitrogen and potash treatments in this experiment consisted of 1,200 lb. ammonium sulphate per acre, and 600 lb. potassium sulphate per acre, applied for two consecutive years. Both these substances would incidentally supply a large concentration of sulphate.

The problem may also be investigated by means of water and sand culture experiments. In preliminary experiments of this sort, cacao seedlings showed definite symptoms of chlorosis and stunted growth in culture solutions containing high concentrations of phosphate. The chlorosis and reduced growth were more marked when the high phosphate cultures also contained a high concentration of sulphate.

No definite explanation of these preliminary tentative results will be attempted at this stage of the investigation. The purpose of this note is merely to present certain theories which indicate the lines on which experiments might be devised in an attempt to provide a definite answer to the questions raised. The preliminary results thus indicate that, under certain conditions, phosphate may indeed interfere with iron uptake by the cacao tree. The experiments now being carried out have been designed to define those conditions, and to provide exact information which may be of considerable practical importance in the proper manuring of the cacao crop.

Summary.

(1) The relationship between soil phosphate and the availability of iron on gypseous cacao soils is discussed.

(2) Cacao trees, growing on phosphate-deficient gypseous soils in Trinidad, fail to respond to the application of phosphatic manures.

(3) The theory is put forward that the application of phosphatic manures to gypseous soils may have a depressing effect on the uptake and utilisation of iron by the cacao tree.

(4) The depressed uptake of iron may be connected with some interaction between phosphate and calcium, or between phosphate and sulphate.

(5) Experiments have been devised to provide definite information on these points. Preliminary tentative results indicate, (*a*) that under certain conditions, the presence of abundant phosphate may markedly depress the uptake of iron by the cacao tree, and (*b*) that this depressed iron uptake may be exaggerated in the presence of a high concentration of sulphate.

(6) The problem appears to be of great practical importance, in view of the high concentration of sulphate in artificial fertilisers, such as ammonium sulphate, potassium sulphate and superphosphate.

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